

ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

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de Alergia e Imunologia

SLaai – Sociedad Latinoamericana
de Alergia, Asma e Inmunología

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The centenary of atopy

O centenário da atopia

Fábio Chigres Kuschnir¹

In 1923, North American immunologists/allergists Arthur F. Coca (1875-1959) and Robert A. Cooke (1880-1959) proposed in their famous study a classification for the different forms of hypersensitivity, which were not well characterized clinically and had ill-defined terminology. By assigning a group for respiratory disorders such as asthma and “hay fever,” they introduced the concept of atopy (from the Greek *atopos* – “out of place”), promoting an innovative advance in the understanding of allergic phenomena. This classification is still used today.^{1,2}

The discoveries and scientific concepts of the past, such as Coca and Cooke's “atopy,” as well as those of today will always be subject to reinterpretations, but one reality remains unchanged over time: despite advances in understanding the mechanisms of allergic diseases and immunological disorders, the increasing number of patients with these conditions will continue to require more and new treatments.

Remembering and commemorating the historical milestones and scientific advances of our specialty is one of the objectives of the Associação Brasileira de Alergia e Imunologia (ASBAI), which was created 50 years ago and is a living part of this history.

As a scientific association, one of our main objectives is to disseminate the most relevant topics in allergy and immunology in an up-to-date and evidence-based way to our associates, to other institutions and medical and health societies, and to society as a whole.

In this sense, the Asthma, Allergy and Immunology Archives (AAAI), the official publication of ASBAI and the Latin American Society of Allergy, Asthma and Immunology (SLAAI), has been fulfilling its role with excellence.

Launched in 2017, the AAAI receives more and more articles each year, written by colleagues from different institutions around the world and whose quality and diversity progressively increase. The availability of articles in Portuguese and English, and their indexing in databases such as LILACS and, more recently, Google Scholar, have increased international interest in our journal and consequently increased the number of downloads and citations of published articles.

Teamwork is critical for the success of the AAAI. In addition to the Boards that preceded us, I would like to highlight the work of our Editor, Pedro Giavina-Bianchi, for his vision and tireless pursuit of the growth of our scientific journal. I would also like to thank the Deputy Editor, Fernando Aarestrup, the Associate Editors, and our competent Editorial Board, as well as all AAAI reviewers. It should be noted that they all work voluntarily for ASBAI.

The AAAI represents the scientific credibility of ASBAI and the strength, richness, and diversity of Brazilian specialists in allergy and clinical immunology.

We hope you enjoy this issue of the AAAI, which contains a Special Article on an objective approach to

1. Chair of Associação Brasileira de Alergia e Imunologia (ASBAI) - 2023-2024.

vaccine reactions conducted by the ASBAI Scientific Department of Immunizations.³

One of the Review Articles addresses the important topic of vaping device use and risk of asthma among adolescents⁴; and, among the Original Articles, we highlight the study that won the Osvaldo Seabra Award for Free Papers at the 2022 ASBAI Congress.⁵

Working in an association is a collective and shared experience, and it is with immense pleasure that the 2023-24 Board invites everyone to continue building the history of ASBAI.

Until 2123!

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Hypersensitivity reactions to vaccines

Reações de hipersensibilidade a vacinas

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ABSTRACT

The expansion of vaccine use and development in recent decades has contributed to the control and eradication of infectious diseases, causing a major impact on public health worldwide. Vaccine safety analysis, which involves careful processes and clinical study, is one of the essential pillars of regulatory approval and use in the population. In current terminology, events supposedly attributable to vaccination and immunization (ESAVI) are defined as any unwanted medical occurrence after vaccination that may or may not have a causal relationship with vaccines or other immunobiologicals. It is noteworthy that rare or unexpected adverse events, including hypersensitivity, can occur during the post-marketing phase, when vaccines are administered to millions of people. In this article, we will discuss the main aspects of post-vaccine hypersensitivity events of interest to specialists and challenges to recognizing the causal agent and appropriate clinical practice. Potential allergens in routine vaccines will also be reviewed to help health professionals identify patients with a potential risk of ESAVI due to such components. Updating health professionals' knowledge about the safety and benefits of vaccines, particularly in special populations, can contribute to more appropriate clinical practice regarding immunization, reducing the risk of exposure to possible allergens in people with allergies to vaccines or their components, avoiding unnecessary contraindications in coincidental or non-serious events.

Keywords: Hypersensitivity, vaccines, immunization.

RESUMO

O desenvolvimento e a ampliação do uso das vacinas durante décadas contribuíram para o controle e erradicação de doenças infecciosas, causando um grande impacto na saúde pública no mundo. A análise de segurança das vacinas percorre criteriosos processos e fases dos estudos clínicos, um dos pilares essenciais para aprovação regulatória e utilização do produto na população. O evento supostamente atribuído à vacinação e imunização (ESAVI), terminologia atual, é definido como qualquer ocorrência médica indesejada após a vacinação que possui, ou não, uma relação causal com o uso de uma vacina ou outro imunobiológico. Cabe ressaltar que eventos adversos mais raros ou inesperados, incluindo os eventos de hipersensibilidade, poderão ocorrer na fase pós-comercialização, quando as vacinas são aplicadas em milhões de pessoas. Neste artigo, serão discutidos os principais aspectos relacionados aos eventos adversos de hipersensibilidade pós-vacinais de interesse do especialista, e os desafios frente ao reconhecimento do agente causal e conduta a ser adotada. Além disso, serão revisados os potenciais alérgenos presentes nas vacinas de uso rotineiro para auxiliar o profissional de saúde na identificação de pacientes com potencial de risco de ESAVI por tais componentes. A atualização do conhecimento acerca da segurança e dos benefícios das vacinas pelos profissionais de saúde, sobretudo em populações especiais, contribui para condutas em imunização mais apropriadas, reduzindo o risco de exposição a um possível alérgeno em pessoas comprovadamente alérgicas às vacinas ou a alguns dos seus componentes, além de evitar contraindicações desnecessárias em eventos coincidentes ou não graves.

Descritores: Hipersensibilidade, vacinas, imunização.

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Introduction

Vaccination is one of the most successful public health interventions. By achieving high vaccination coverage, Brazil has greatly reduced the incidence of vaccine-preventable diseases.¹

All vaccines available in Brazil through the public and private health systems have undergone extensive clinical testing for efficacy and safety before being approved by the relevant regulatory agencies; however, events supposedly attributable to vaccination and immunization (ESAVI) can still occur.^{2,3}

An ESAVI is defined as any untoward medical occurrence which follows vaccination and which may or may not have a causal relationship with the vaccine or other immunobiological agent (immunoglobulin or heterologous sera). Any unfavorable or unintentional event, such as a sign, symptom, illness, or abnormal laboratory finding, can be classified as an ESAVI.^{4,5} An ESAVI may be caused by several factors: I. Factors related to the vaccine product: including type (attenuated or inactivated), strain, culture medium, the inactivation or attenuation process, adjuvants, stabilizers, or preservatives, or a specific batch of vaccine; II. Factors related to the vaccine recipient: age, sex, number of vaccine doses and previous exposure to the vaccine, adverse reactions to previous doses, comorbid conditions, allergic conditions, autoimmunity, immunodeficiency; III. Administration-related factors: needle and syringe, site of inoculation, route of inoculation (intradermal, subcutaneous, or intramuscular).⁵

The cumulative experience acquired throughout the development of the National Immunization Program (NIP) has allowed Brazil to compile extensive knowledge about ESAVI, which can be classified as expected or unexpected, depending on their nature and the characteristics of the agent to which they are supposedly attributable. Among expected events,

some are relatively trivial, such as fever, pain, and local swelling, while others can be severe, such as febrile seizures, hypotonic-hyporesponsive episode (HHE), anaphylaxis, etc. Unexpected events are those not previously identified, such as intestinal intussusception after administration of the rhesus rotavirus-based human rotavirus vaccine, or those known to occur but only rarely seen with older vaccines, such as viscerotropic disease and multiorgan failure after the yellow fever (YF) vaccine. Unexpected events also include those arising from issues related to the quality of the vaccine product, such as batch contamination (which can cause localized abscesses) or, in certain vaccines, presence of endotoxin (leading to febrile reactions and sepsis-like symptoms).⁵

Adverse events can also occur coincidentally after vaccine administration, in which case they are not directly related to the biological effects of the vaccine produce (i.e. they would have occurred even in the absence of vaccination).⁶

ESAVI can be further classified as serious or non-serious. *Serious* adverse events (SAEs) are those that require hospitalization for more than 24 hours or prolong an already existing hospitalization, cause significant dysfunction and/or persistent disability, result in a congenital anomaly, are life-threatening, and/or lead to death. All other events not meeting the SAE criteria are considered *non-serious*.⁵

Another important feature is whether reactions are *local* or *systemic*. Local reactions such as edema, erythema, and tenderness at the injection site are common after the administration of some vaccines. These symptoms do not contraindicate subsequent vaccinations.⁷ Systemic reactions are mostly mild and can range from fever, myalgia, irritability, anorexia, drowsiness, persistent crying, and headache to more moderate and (rarely) severe events, such as

anaphylaxis, seizures, hypotonic-hyporesponsive episodes, and (with the yellow fever vaccine) acute viscerotropic and acute neurotropic disease.

This article will address the main aspects related to those adverse events following immunization of interest to the allergist/immunologist, the challenges posed by recognition of the causal agent, and key tenets of patient management. We will also review the main allergens present in routinely used vaccines to help clinicians identify patients at potential risk of ESAVI caused by these components.

Type I hypersensitivity reactions

Type I hypersensitivity reactions are mediated by preformed IgE antibodies against vaccine components. They rarely occur, but are considered potentially serious adverse events that require investigation and a keen understanding of the risk–benefit ratio for the administration of subsequent doses.⁷ These reactions can occur after administration of any vaccine or serum, especially those of nonhuman (equine) origin.

Typically, reactions occur immediately or within 4 hours after exposure to the allergen, and can manifest as cutaneous or systemic symptoms (anaphylaxis).^{7,8} The estimated rate of anaphylaxis ranges from 1 in 100,000 to 1 in 1,000,000 doses for most commonly administered vaccines.^{9–11} One study evaluated cases of anaphylaxis reported to the United States Vaccine Adverse Event Reporting System (VAERS) from 1990 to 2016 and identified 8 fatal cases, of which 4 had no personal history of hypersensitivity.¹²

Another study showed the incidence of anaphylaxis to vaccines to be 1.31 cases per 1,000,000 vaccine doses administered (95% CI 0.90–1.84). Although anaphylaxis is a potentially serious reaction, it is treatable and recovery can be achieved with no sequelae or fatal outcome.⁸

During investigation of a potential allergic reaction to a vaccine, a detailed history is essential to rule out other etiologies unrelated to the vaccine components (e.g. exposure to latex in latex-allergic patients, anti-inflammatory medication).

The leading cause of immediate adverse reactions to vaccines is gelatin. Other components present in certain vaccines may also be involved in reactions, such as vaccine antigens, carrier proteins, egg, milk, yeast, latex, antimicrobials, and polyethylene glycol.^{8,13} Allergic reactions to vaccines can be due to the active ingredient (the antigen) or to other

components.^{7,8} Vaccine antigens may consist of the microorganism in whole or in part, inactivated toxins (toxoids), or both, and are intended to elicit a protective immune response. These antigens are rarely the cause of hypersensitivity reactions. In most cases, vaccine hypersensitivity is actually caused by non-antigen components.⁸ Table 1 summarizes the main vaccine components that can cause IgE-mediated reactions.

Vaccine components and immediate hypersensitivity reactions

Egg

Some commonly used vaccines contain small amounts of residual egg protein (ovalbumin) from the manufacturing process. Ovalbumin concentrations are generally higher in vaccines grown in embryonated chicken eggs (influenza, yellow fever, and rabies vaccines) and lower in those grown in chick embryo fibroblast cells, such as the measles/mumps/rubella (MMR) vaccine.⁸

The MMR vaccine is considered safe for patients with egg allergy, as it contains such small amounts of egg protein it is incapable of eliciting an allergic response.^{7,13,14} Several studies have proven the safety of the vaccine in egg-allergic patients, and there is no contraindication to its use.^{7,14,15} Most anaphylactic reactions to MMR vaccine have been attributed to gelatin allergy.⁷

The influenza vaccine was contraindicated in patients with egg allergy for many years, but it is now known to be safe for this patient population as well. A review of 28 studies in which influenza vaccine was administered to 4,315 egg-allergic patients, of which 656 had a history of anaphylaxis to egg, found no serious reactions to the vaccine.¹⁶ Furthermore, in studies that included non-egg-allergic controls, the rate of mild reactions was similar in controls and in those with allergies.¹⁶ Therefore, current recommendations hold that the influenza vaccine can be given to egg-allergic patients.^{7,8}

The yellow fever vaccine contains higher amounts of protein, and whether or not it is indicated should be evaluated cautiously. Furthermore, the yellow fever vaccine is not heated at any time during the manufacturing process, so even patients who tolerate boiled/fried eggs may react to the vaccine.¹⁷ A Brazilian study evaluated the safety profile of administering this vaccine to egg-allergic patients. In the study protocol, all patients underwent a skin prick test with undiluted

vaccine. If negative, an intradermal (ID) test was performed with the vaccine at a dilution of 1:100. If the ID test was negative, a full dose of the vaccine was given. If either the skin prick test or ID test was positive, the vaccine was applied in fractional (graded) doses according to the desensitization protocol. All 58 patients included in the study had a negative skin prick test; 10 had a positive ID test and were desensitized. Of these 10 patients, 4 had no hypersensitivity reaction whatsoever, 4 had local reactions (hives at the application site), and 2 had urticaria with subjective symptoms. Of the 48 remaining patients with a negative ID test, none had any reaction to the vaccine, which was given as a single dose.¹⁸ Therefore, egg-allergic patients in whom yellow fever vaccination is

indicated should be referred to a specialist for skin testing and, if necessary, desensitization.¹⁸

Milk

Patients with cow's milk allergy (CMA) should not receive the MMR vaccine manufactured by the Serum Institute of India Ltd., which may contain alpha-lactalbumin. Studies in Brazil and Chile reported cases of allergic reactions after exposure to this vaccine in patients with CMA.^{19,20} In Chile, 9 cases of immediate hypersensitivity reactions after MMR vaccine were reported in milk-allergic patients. All had a diagnosis of CMA before administration of the first dose of the MMR vaccine, but none had a history of anaphylaxis.

Table 1

Major vaccine components associated with IgE-mediated reactions

Component	Vaccine	Recommendation
Egg	MMR	Administer vaccine without special precautions
	Influenza	Administer vaccine; no special precautions required
	Yellow fever	Assess on case-by-case basis. Consider skin testing with vaccine in patients with a history of anaphylactic reactions to eggs. If positive, administer in graded doses
Milk	MMR	MMR vaccine manufactured by the Serum Institute of India Ltd. is contraindicated; administer vaccine from any other manufacturer that does not contain milk
	DTaP	No contraindication. In patients with severe, low-threshold CMA, assess on case-by-case basis
	Tdap	
Gelatin	Influenza, MMR, rabies, varicella, herpes zoster	Consider skin testing with vaccine. If positive, administer in graded doses
Yeast	Hepatitis B	Consider skin testing with vaccine
	Quadrivalent HPV	If positive, administer in graded doses
Latex	http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/latex-table.pdf	Administer vaccine. Vaccinator must not wear latex gloves

All cases occurred within 2 hours of administration of Serum Institute of India MMR vaccine; 6 had anaphylaxis, 2 had only respiratory manifestations, and 1 developed only cutaneous manifestations.²⁰ Therefore, patients with CMA should receive MMR vaccine made by other manufacturers which does not contain milk proteins.

Milk proteins can also be used as a growth medium for tetanus-pertussis-diphtheria vaccines (DTaP and Tdap). A case series of 8 patients with severe CMA reported anaphylactic reactions with DTaP or Tdap vaccines. The study found that these vaccines contained casein derivatives, which could potentially explain the severe, immediate reactions elicited in patients with CMA.²¹ However, the methodology used to identify the causality of the described reactions is controversial. Therefore, according to an EAACI position statement, vaccination with DTaP and Tdap vaccines is not considered to contribute to the pathogenesis of allergic diseases, and atopy does not contraindicate administration of these vaccines.²²

Patients with IgE-mediated ALV, especially those with the severe form and with a low threshold for development of reactions, should be aware of medications and vaccines that potentially may contain traces or even minimal amounts of cow's milk protein. In these cases, an individual risk assessment is recommended, and vaccination in a supervised environment may be indicated.

Gelatin

Gelatin is added to vaccines – most often, attenuated virus vaccines – as a stabilizer, and is the component primarily responsible for most allergic reactions.⁷ Studies have shown that gelatin was the triggering agent of anaphylactic reactions to the MMR²³⁻²⁵ and chickenpox^{26,27} vaccines.

A previous history of reaction to gelatin must be evaluated before indicating vaccines which contain this component, but the absence of such a history does not rule out the possibility of a reaction to these vaccines, since oral intake of gelatin allows digestion of gelatin into less allergenic peptide fragments.⁷ Patients with alpha-gal allergy should also be approached with caution.⁸

Patients with a known history of allergy after oral gelatin intake or administration of a vaccine containing gelatin and who have indications for a vaccine containing this component should undergo testing. The workup may include gelatin-specific

IgE measurement, a skin prick test with undiluted vaccine, and, if negative, an ID test with the vaccine at 1:100 dilution.⁷ If both skin tests are negative, the vaccine can be given as a single dose with no further precautions except observation for 30 to 60 minutes after administration. If the skin tests are positive, a graded-dose can be administered in a facility equipped to respond in case of anaphylaxis.⁷

Yeast

The antigens of several vaccines, including hepatitis B, HPV, and one of the conjugated meningococcal vaccines, are recombinant proteins expressed in *Saccharomyces cerevisiae*.⁸ In a review, DiMiceli et al. identified 180,895 adverse events, 15 of which were reports of probable or possible anaphylaxis, after vaccination in patients with a history of yeast or fungal allergy. Of these patients, 11 had received hepatitis B vaccine that contained traces of yeast protein, but in these cases no tests were performed to prove sensitization to the culprit protein.²⁸ Therefore, allergic reactions to vaccines allegedly caused by yeast or fungi are exceedingly rare. In some cases, skin testing may be performed in patients with a history of *S. cerevisiae* allergy; if positive, supervised administration of graded doses of the potentially yeast-containing vaccine may be considered.⁸

Latex

Exposure to latex during vaccination may be related to the packaging of the vaccine vial or syringe, but allergic reactions to such exposures are exceedingly rare. Unlike balloons and gloves, from which the latex allergen can be easily eluted, this component cannot be eluted from vaccine packaging. It is thus recommended that vaccinators wear latex-free gloves while administering vaccines to patients with latex allergy.⁷ In case of multidose vials, one suggestion is to administer the first dose from a fresh vial to the latex-allergic patient.

Antimicrobials

Several antimicrobial agents, such as gentamicin, tetracycline, neomycin, streptomycin, and polymyxin B, are used to prevent bacterial or fungal growth during the vaccine manufacturing process. During the purification process, most of these antimicrobials are removed; although traces of these agents may still be present in some vaccines, they rarely cause

systemic reactions. People with a history of severe allergy (anaphylaxis) to antimicrobial agents contained in a given vaccine may benefit from an individualized immunization approach, and either replacement of the immunogen or vaccination under supervision may be considered.⁸

Polyethylene glycol (PEG) and Polysorbate 80 (PS80)

Polyethylene glycol, used as a stabilizer in drugs, cosmetics, and other product formulations, has recently been implicated as a possible cause of anaphylactic reactions following administration of COVID-19 mRNA vaccines. However, the evidence supporting this hypothesis is contradictory, and other factors may be involved in these immediate reactions.²⁹

One study correlated an episode of anaphylactic reaction after administration of the Pfizer/BioNTech vaccine with the presence of anti-PEG antibodies, as determined by immediate-reading skin tests. However, the study also found that three other participants who experienced similar systemic adverse events did not have anti-PEG antibodies. Despite the small number of cases described in this study, the authors suggest that the role of PEG in eliciting anaphylactic reactions may be limited. Continuous monitoring of data on the rare potential risk of anaphylaxis and active surveillance of the population receiving COVID-19 vaccines may help identify risk factors for the development of severe allergic reactions following immunization. It bears stressing that people with a history of proven, severe allergy to PEG should not receive any vaccines containing this component. A better understanding of the frequency, characteristics, and mechanisms of reactions to PEG may aid in the development of increasingly safer vaccines for allergic patients.²⁹

Polysorbate 80 (PS80) is a molecule structurally related to PEG which can be found in some vaccines. Some adverse events (including anaphylaxis) after vaccination with products containing PS80 have been described in the literature, which has led researchers to hypothesize a role for PS80 in inducing anaphylaxis.^{30,31}

Adverse events consistent with type II (cytotoxic) hypersensitivity reactions

Type II hypersensitivity reactions are characterized by the formation of antibodies that attach to body cells,

resulting in cell destruction by complement proteins and by lymphocytes that bind to the antibodies. This mechanism is probably involved in the demyelination (destruction of the nerve myelin sheath) that can occur after administration of certain live virus vaccines or nerve-tissue rabies vaccine, causing conditions such as acute disseminated encephalomyelitis (ADEM) or the Guillain-Barré syndrome (GBS). The yellow fever, influenza, DTP, and meningococcal vaccines have all been temporally associated with these reactions in the literature.⁵

Although cases of vaccine-associated idiopathic thrombocytopenic purpura (ITP) are rare, clinicians should bear in mind that the risk of ITP is increased for up to 6 weeks after MMR vaccination. In a 2010 systematic review, the median incidence of ITP after MMR vaccine administration was 2.6 cases per 100,000 doses given. This incidence is lower than that caused by natural measles and rubella infection, and similar to the incidence of ITP in the general population.³²

Adverse events consistent with type III (immune complex) hypersensitivity reactions

These reactions are caused by the formation of immune complexes, which lead to vasculitis and tissue necrosis at the injection site (as can occur after multiple diphtheria-tetanus vaccine boosters – the so-called Arthus phenomenon) or to generalized manifestations, as in serum sickness. Arthus reactions have been reported after the administration of tetanus, diphtheria, hepatitis B, 23-valent pneumococcal, and meningococcal conjugate vaccines.³³

Management of Arthus reaction is based on topical corticosteroids (to relieve discomfort caused by itching and rash), antihistamines (which also help relieve rash and itching), and non-steroidal anti-inflammatory drugs (to relieve joint pain when present).⁵

Serum sickness, characterized by the onset of rash, arthralgia or arthritis, fever, lymphadenopathy, and anorexia approximately 1 to 2 weeks after exposure to the antigen, may manifest after rabies vaccination and, more rarely, after administration of influenza, tetanus, and pneumococcal vaccines.³⁴

Adverse events consistent with type IV (delayed) hypersensitivity reactions

Type IV hypersensitivity reactions are inflammatory reactions triggered by mononuclear leukocytes. The

term “delayed” is used to differentiate a secondary cellular response, which develops 48-72 hours after allergen exposure, from an immediate hypersensitivity response, which generally develops within 12 minutes. These reactions are mediated by T cells and monocytes/macrophages, not antibodies.⁵

Delayed hypersensitivity reactions can be localized, as in individuals who develop skin reactions to the neomycin and thiomersal used as preservatives in some vaccines.⁵

Vaccines containing aluminum adjuvants can induce formation of chronic subcutaneous nodules at the injection site.³⁵ Diphtheria-tetanus-pertussis vaccines (alone or in combination with other antigens), as well as human papillomavirus and hepatitis A and B vaccines, may contain aluminum. The risk of this complication is estimated at around 0.03% to 0.83%. Nodules usually appear approximately 3 months after vaccination, and can persist for many years (up to 3-4 years). Severe itching, local eczema, hypertrichosis, and discoloration may be present at the nodule site. Contact sensitivity to aluminum can be confirmed in clinical practice through patch testing.³⁶

Severe manifestations such as the Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and acute generalized exanthematous pustulosis have been reported as adverse events following immunization, although these reactions are vanishingly rare. The mechanisms putatively involved in delayed post-vaccination reactions are not well established, although preexisting allergy to vaccine components or possible genetic variations in antigen presentation or processing may be predisposing factors for the development of clinically severe reactions.³¹

Vaccines related to hypersensitivity events

Yellow fever vaccine

The yellow fever (YF) vaccine is grown in embryonated chicken eggs. Due to the manufacturing process, residual amounts of egg protein may be present in this vaccine. Two YF vaccines are available in Brazil. The amount of ovalbumin varies, ranging from 2.43 to 4.42 µg/mL depending on the batch. In addition to ovalbumin, the vaccine agent itself (live attenuated yellow fever virus), bovine gelatin, erythromycin, kanamycin, and latex (the last four being present only in some formulations) are potentially causative of hypersensitivity reactions.⁵

Inquiring as to severe allergy after ingestion or contact with eggs is considered appropriate for persons with a history of other severe allergies who will receive the yellow fever vaccine. For children without any clinical history consistent with egg allergy and in whom complementary feeds are being introduced, there is no evidence of a need to try eggs prior to yellow fever vaccination. Likewise, there is no recommendation for egg-specific IgE testing in children without a history of allergy after oral egg intake before the vaccine is administered.³⁷

Individuals with a known diagnosis or clinical suspicion of egg allergy should be referred to a specialist for investigation of possible egg sensitization using immediate-reading skin tests or allergen-specific serum IgE. If these tests are negative, the vaccine can be administered at the standard dose and the individual kept under observation for 30 to 60 minutes. If a diagnosis of egg-mediated IgE allergy is confirmed by clinical or laboratory criteria by the allergist, the patient can receive the YF vaccine through desensitization protocols or fractional dosing, in an appropriate setting for the management of possible anaphylaxis.^{16,17,37}

An important aspect to be considered is whether the patient reports tolerating boiled or fried eggs, but is not known to tolerate raw eggs. The YF vaccine is not subjected to high temperatures at any time during its manufacturing process, so even a patient who tolerates boiled or fried eggs may develop an immediate reaction after vaccine administration.³⁷

Influenza vaccine

Influenza vaccine is grown in allantoic fluid from embryonated chicken eggs and contains varying amounts of ovalbumin depending on the manufacturer. In general, currently marketed influenza vaccines contain less than 1.2 µg/mL ovalbumin.

Egg allergy was once considered a contraindication to influenza vaccination, but studies including 4,300 individuals with a history of egg allergy, of which 650 reported anaphylactic reactions, showed good tolerance to the vaccine. The current recommendation is that individuals with a history of severe allergy (anaphylaxis) to chicken eggs receive the influenza vaccine and remain under observation for 30 to 60 minutes. The American Academy of Pediatrics (AAP) advises that influenza vaccination in egg-allergic patients is safe and does not require additional precautions, since there is already a

formal recommendation on the need for standard precautions for all patients who receive any type of immunogen.³⁸

MMR (measles, mumps, rubella) vaccine

The MMR vaccine contains negligible amounts of egg protein. In many studies, individuals with egg allergy – even those with a history of severe hypersensitivity – had an accordingly negligible risk of anaphylactic reactions. Skin testing with the MMR vaccine is not recommended, as it has no established predictive value.¹⁶ Many reactions attributed to the MMR vaccine are possibly due to other components, such as gelatin.³⁷

In 2014, some cases of children with CMA who developed anaphylaxis after receiving MMR vaccine manufactured by the Serum Institute of India were reported.^{19,20} Upon investigation, the vaccine was found to contain traces of lactalbumin. Therefore, the Brazilian Ministry of Health advises that children with CMA receive MMR vaccine from another manufacturer that does not contain milk proteins; if a substitute vaccine is not available, vaccination should be deferred.³⁹

Varicella vaccine

The varicella zoster (chickenpox) vaccine is a live attenuated virus vaccine (Oka strain) and may contain gelatin and traces of neomycin. The estimated rate of anaphylaxis to varicella vaccine reported to VAERS from 2006 to 2016 was 1.2 per 1 million doses deployed.^{7,12} Ozaki et al. described 28 severe anaphylactic reactions and 139 non-severe allergic reactions following immunization with gelatin-containing varicella vaccine from 1994 to 1999 in Japan, out of 1.41 million doses administered (11.6/100,000). All nine available serum samples from children with anaphylaxis were gelatin-specific IgE-positive, whereas 55 of 70 available sera from children with non-severe allergy were positive. On the other hand, 5 non-serious allergic reactions and no serious reactions were described out of 1.3 million doses of gelatin-free varicella vaccine administered from 1999 to 2002 (0.38/100,000). The authors concluded that the gelatin-free vaccine was safer and provided data demonstrating comparable immunogenicity to that of the previous gelatin-containing vaccine.⁴⁰

Serious and non-serious ESAVI reported in the literature for each vaccine are listed in Table 2.

Pneumococcal vaccines

Streptococcus pneumoniae is the leading cause of morbidity and mortality among children worldwide. It is the most common cause of otitis and invasive bacterial disease (including pneumonia, bacteremia, and meningitis) in children.⁷

More than 90 pneumococcal serotypes have been identified, but approximately 20 serotypes account for most infections worldwide.⁸

Two types of pneumococcal vaccine are currently commercially available in Brazil: the 23-valent polysaccharide vaccine (PPSV), available since the 1980s, and the 10- and 13-valent pneumococcal conjugate vaccines (PCV).³⁷

The 23-valent pneumococcal polysaccharide vaccine covers the 23 most prevalent pneumococcal serotypes, and contains phenol as a preservative. The 13-valent pneumococcal conjugate vaccine contains polysorbate 80. In the 10-valent pneumococcal conjugate vaccine, the conjugate is nontypeable *Haemophilus influenzae* lipoprotein D for eight pneumococcal serotypes and the CRM₁₉₇ protein for the remaining two. Overall, pneumococcal vaccines are well tolerated, with more local adverse events being reported.^{41,42}

Two cases of anaphylaxis associated with the 23-valent pneumococcal polysaccharide vaccine were reported in children, in 2001 and 2010; in both, immediate-read skin tests demonstrated sensitization to pneumococcal polysaccharides. These cases highlight that hypersensitivity reactions to the vaccine antigen are rare events, and their confirmation is challenging in clinical practice.⁴³

The first case of anaphylaxis to the 13-valent pneumococcal conjugate vaccine reported in the literature occurred in a 1-year-old child who received several vaccines at the same visit. Investigation by a specialist with skin testing and a basophil activation test concluded that CRM₁₉₇ was the causative agent of anaphylaxis.⁴⁴

Adult diphtheria toxoid-tetanus toxoid vaccine adsorbed (dT)

The adult diphtheria-tetanus vaccine is composed of diphtheria toxoid, tetanus toxoid, aluminum hydroxide, thiomersal, and buffered saline solution (pH 6.4). Hypersensitivity reactions are rare, with the majority being caused by thiomersal and aluminum.⁵

Table 2
Adverse events by vaccine

Vaccine	Adverse event			Hypersensitivity reaction
	Site	General	Specific	
Hepatitis B	Pain, induration, redness	Fever, irritability, fatigue, headache	ITP	Yes
DTP-HB/Hib	Pain, induration, redness	Fever, irritability, fatigue, headache, drowsiness	Apnea, encephalopathy, seizures, persistent crying, HHE	Yes
OPV		Rash, urticaria	Acute flaccid paralysis from vaccine-derived poliovirus, aseptic meningitis	Yes
Rotavirus		Nausea, vomiting	Intestinal intussusception	
Yellow fever	Pain, redness, induration	Fever, myalgia, headache	Encephalitis, acute viscerotropic disease, acute neurotropic disease	Yes
MMR (Measles, Mumps, Rubella)	Burning, hyperesthesia, redness, abscess, lymphadenopathy	Fever, headache, irritability, rash	Encephalitis, meningitis, subacute sclerosing panencephalitis, Guillain-Barré syndrome, orchitis, parotitis, ITP	Yes
IPV	Redness, induration, pain	Fever		Rarely
Hepatitis A	Redness, induration, pain	Fever, headache, malaise, fatigue		Rarely
Varicella	Redness, induration, pain	Fever, mild vesicular rash	Reactions are more common in immunosuppressed patients	Rarely
Pneumococcal, 10-valent	Redness, induration,	Irritability, drowsiness, excessive crying	Rarely	
Pneumococcal, 23-valent	Redness, edema, pain	Fever, myalgia, arthralgia, headache, listlessness	Local manifestations are more frequent in revaccinated patients	Rarely
Meningitis C	Redness, induration, pain	Fever and irritability		Rarely

ITP = idiopathic thrombocytopenic purpura.

HHE = hypotonic-hyporesponsive episode.

Vaccines with a pertussis component

Diphtheria-tetanus-pertussis vaccine acellular (DTaP)

Most allergic reactions to vaccines are reported in patients immunized against tetanus, diphtheria, and pertussis.⁴⁵ However, hypersensitivity reactions are exceedingly rare, at a rate of 2 per 1 million doses of DTaP.³⁵ When they do occur, they are often allergic reactions such as urticaria, or, in more severe cases, anaphylaxis.⁴⁵

In addition to the active ingredients (diphtheria toxoid, tetanus toxoid, components of the *Bordetella pertussis* bacterial capsule, hemagglutinin, pertactin, phenoxyethanol), these vaccines contain excipients which vary (including in concentration) by manufacturer, and are the leading causes of allergic reactions. The excipients are: aluminum hydroxide, aluminum phosphate, sodium chloride, and water for injection. Traces of formaldehyde, polysorbate 80, glutaraldehyde, and glycine are likewise present.⁵

Aluminum may be present both as an excipient and as an adjuvant, to enhance the immunogenicity of the antigens, and may induce type IV hypersensitivity (contact allergy). Therefore, caution is warranted in individuals with a history of hypersensitivity to aluminum (most commonly to antiperspirants).³ On rare occasions (< 1%), vaccinated children may develop pruritus and nodule formation at the application site due to the aluminum contained in the vaccine. The cause of this phenomenon is still poorly defined.²² Contact hypersensitivity has been demonstrated in 77% of children with pruritic nodules and in 8% of asymptomatic siblings who received the same vaccines.³⁵ Subcutaneous nodules can develop and persist for months or years before gradually disappearing. Risk factors for aluminum sensitization during vaccination appear to be the amount of aluminum in the vaccine, the number of vaccinations administered, and the type of aluminum component, with aluminum hydroxide being more likely to induce sensitization than aluminum phosphate.²²

Many manufacturers include glycine (gelatin) in their vaccines, which increases allergenic potential. The correlation of previous administration of DTaP doses with the development of gelatin hypersensitivity has been investigated, as it could explain the development of allergy to other glycine-containing vaccines, such as MMR. Most anaphylactic and urticarial reactions attributed to MMR are in fact

related to elevated levels of gelatin-specific IgE, not to the vaccine antigens themselves.⁴⁶

Whole-cell diphtheria-tetanus-pertussis vaccine (DTP)

The DTP vaccine, which contains whole *Bordetella pertussis* cells, is associated with a higher frequency of adverse reactions compared to the DTaP vaccine. However, the adjuvant composition of the two vaccines is similar, and the risk of allergic reactions may be the same. In some countries, whole-cell DTP vaccine does not contain aluminum⁴⁷; therefore, allergic reactions are reported less frequently than with the acellular formulation.

DTaP-IPV-HepB+Hib (hexavalent) vaccine

The hexavalent vaccine is composed of diphtheria toxoid, tetanus toxoid, pertussis toxoid, filamentous hemagglutinin, pertactin, hepatitis B surface antigen, poliovirus, and *Haemophilus influenzae* type b polysaccharide as active ingredients. Hydrated aluminum hydroxide is the sole adjuvant. The excipients are lactose, sodium chloride, Medium 199 (M-199), aluminum hydroxide, aluminum phosphate, and water for injection. Traces of potassium chloride, disodium phosphate, monopotassium phosphate, polysorbate 20 and 80, glycine, formaldehyde, neomycin sulfate, and polymyxin B sulfate may be present. Local reactions to antibiotics do not contraindicate vaccination. Reactions to aluminum and glycine are similar to those seen with DTaP.³⁷

DTaP-IPV+Hib (pentavalent) vaccine

The active components of the pentavalent vaccine are diphtheria toxoid, tetanus toxoid, *Bordetella pertussis* antigens, pertussis toxoid (PT) 25 µg, filamentous hemagglutinin, pertactin, poliovirus type 1 strain Mahoney inactivated (IPV), *Haemophilus influenzae* type b polysaccharide (polyribosylribitol phosphate), and aluminum hydroxide as adjuvant. The excipients are lactose, sodium chloride, aluminum salts, medium 199 (M-199), and water for injection. Traces of potassium chloride, disodium phosphate, monopotassium phosphate, polysorbate 80, glycine, formaldehyde, neomycin sulfate, and polymyxin B sulfate may be present. Again, local reactions to antibiotics do not contraindicate vaccination.⁴⁶ Reactions to aluminum and glycine are similar to those seen with DTaP.

In a prospective study of 4758 children, 0.66% (n = 38) developed pruritic granuloma after administration of the pentavalent vaccine Pentavac® (DTaP-Hib-IPV). Patch testing for elemental aluminum and 2% aluminum hydrochloride hexahydrate has been suggested, but some cases may not be diagnosed and sensitization may fail to be identified. An alternative to improve the sensitivity of the test would be to use 10% aluminum hydrochloride hexahydrate as the antigen. To help confirm the diagnosis of hypersensitivity, it is recommended that patch testing performed with 10% aluminum hydroxide be read at 3 or 4 days and at 1 week.⁴⁷

COVID-19

In the current coronavirus disease 2019 (COVID-19) pandemic, vaccines have been one of the most promising measures for controlling the spread of the virus and reducing severe cases and deaths. COVID-19 vaccines were developed using several different technologies, leveraging vast experience with previous platforms and due to the massive social and economic impact of the pandemic. The most common COVID-19 vaccines in use worldwide are inactivated virus, recombinant viral vector, nucleic acid (mRNA), virus-like particle, and protein subunit vaccines. Other vaccine platforms, such as attenuated virus and DNA vaccines, are at various stages of development.⁴⁸

Anaphylaxis-like reactions following administration of the Moderna and Pfizer/BioNTech mRNA COVID-19 vaccines have been reported in the UK and U.S. The incidence of these reactions suggests that, while still rare, the rate may be higher with these vaccines than previously reported in the literature, at approximately 2.5 to 4.7 events per 1 million doses.⁴⁹

Some cases characterized and treated as anaphylaxis subsequently resolved, and patients received the second dose of the vaccine uneventfully.⁵⁰ Other cases have been described that clearly resemble anaphylactic reactions, with rapid onset of symptoms and need for endotracheal intubation, although no investigation of the mechanism has been described.⁴⁹

Occasionally, patients develop hives with or without mild angioedema within hours or days of receiving the vaccine. Most potentially serious systemic allergic reactions to mRNA COVID-19 vaccines reported to date have occurred within 30 minutes of vaccination (the majority within 15 minutes). This rapid onset of

symptoms can assist recognition of anaphylaxis, and determines which precautionary measures must be taken at the vaccination facility.³⁷

So-called persistent intermittent delayed swelling (PIDS) of the face in areas previously injected with cosmetic dermal fillers has been reported following administration of the Moderna mRNA vaccine. The pathophysiology remains unclear and may not involve allergic mechanisms, although local inflammation has been observed.⁵¹

Delayed local reactions are rare events and have been described with several vaccines. In phase III studies of the Moderna mRNA-1273 vaccine, delayed local reactions were seen in 0.8% of patients after the first dose and in 0.2% after the second dose.⁵² Blumenthal et al. described 12 patients with extensive late local reactions after administration of the mRNA-1273 vaccine. Median time to reaction onset was 8 days (range, 4 to 11 days) after the first dose. Some patients had concomitant systemic symptoms, and two patients had other skin manifestations. Most patients were treated symptomatically (e.g. local ice and antihistamines). Symptoms resolved within a median of 6 days after onset (range, 2 to 11 days). All patients received the second dose of the vaccine, as this type of reaction does not contraindicate subsequent vaccination. Although half of the patients had no reaction, three patients had reactions similar to that observed after the first dose, and three patients had recurrence of the local reaction to a lesser degree compared to the first-dose reaction.⁵³

Most reactions have occurred with the first dose, which suggests either that the patients were previously sensitized to some vaccine component or that the mechanism involved in these reactions could be different from known hypersensitivity mechanisms. As noted above, most allergic reactions to vaccines are usually not due to the antigen component, but rather to excipients. Table 3 lists available COVID-19 vaccines with their types, components, and hypersensitivity reactions reported up to January 2021.

Immediate hypersensitivity reactions have been more frequent with mRNA vaccines than with other commonly used vaccines, as described above. Therefore, efforts have been made to understand the mechanisms implicated in hypersensitivity to these vaccines. The Pfizer-BioNTech and Moderna vaccines do not contain any food or drug components nor latex, but both contain polyethylene glycol (PEG), which is used as an excipient with the aim of stabilizing the mRNA-containing lipid nanoparticles. The specific

type of PEG used in these vaccines was different from the PEG used in most other commercial products. Countless products contain PEG (also known as macrogol), including medicines, cosmetics, and foodstuffs.⁵⁴ Despite this widespread use, PEG allergy is very rare. Most reactions to PEG described in the literature are related to high-molecular-weight PEG, with low-molecular-weight PEG (more common in everyday products) being a less frequent cause of adverse reactions. The underlying pathophysiological mechanism of PEG allergy is still unclear, but the presence of anti-PEG IgE has been described in some patients with PEG-induced anaphylaxis. PEG also seems to induce complement activation mechanisms *in vitro*.⁵⁵ Most PEG allergies are usually discovered during evaluation of patients who have apparently had reactions to unrelated products, such as injectable corticosteroids, processed foods, cosmetics, drugs, and other substances.⁵⁴

Polysorbate, like PEG, is an excipient used in multiple drug preparations (including vaccines), creams, lotions, and drug tablets. Polysorbates are derived from PEG, but have a very low molecular weight and are therefore much less likely to trigger an allergic reaction. The Oxford/AstraZeneca (ChAdOx1) vaccine is manufactured using a viral vector (non-replicating chimpanzee adenovirus) and contains histidine, sucrose, sodium chloride, magnesium chloride, polysorbate 80, disodium edetate (EDTA), and ethanol. Polysorbate 80 is present at a concentration of less than 100 µg per dose – the same amount found in other vaccines, such as the influenza vaccine, with which higher rates of allergic reactions have not been observed.⁵⁵

The Janssen (Johnson & Johnson) vaccine also uses viral vector technology (recombinant adenovirus) and contains polysorbate 80. In phase III studies, hypersensitivity reactions (urticaria) were rare (1/10,000 to 1/1,000 doses) and anaphylactic reactions were reported, but the frequency could not be estimated from the available data.⁵⁶

CoronaVac is an inactivated virus vaccine that contains aluminum hydroxide, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, water for injection, and sodium hydroxide. In safety analyses of phase III studies including 5051 participants, 50.8% had adverse events within 7 days of administration, and 40.1% of the reactions were pain at the injection site. Hypersensitivity reactions were rare, occurring at a frequency of 1/1000 to 1/100.^{57,58}

Of the 221 participants of a Turkish trial, 62 (28.1%) reported allergic or skin reactions (injection site pain and/or inflammatory reactions) temporally related to the CoronaVac vaccine. Of the 62 patients who had skin reactions, 25 (11.3%) had no personal or family history of allergy, while the remaining 37 reported a history of allergy. The 25 patients with no personal/family history reported the following events (some patients had more than one reaction): urticaria (n=12, 5.4%); papulosquamous reactions (e.g. pityriasis rosea) (n=8; 3.6%); herpes infection (n=4; 1.8%), consisting of herpes zoster (HZ) (n=2) and herpes simplex (HS) (n=2); angioedema (n=3; 1.4%), type IV hypersensitivity reactions, such as erythema multiforme, lichenoid drug eruption, and drug hypersensitivity syndrome (n=3; 1.4%); palmar erythema (n=2; 0.9%); anaphylaxis (n=1; 0.5%); conjunctivitis (n=1; 0.5%); and small-vessel vasculitis (n=1; 0.5%). However, it should be noted that the events reported in this study were only temporally related to vaccination, with no further investigation or proof of causality.⁵⁹

Rationale for investigation of hypersensitivity reactions following vaccination

When a patient is referred to an allergist/immunologist for assessment of a potential vaccine hypersensitivity reaction, the clinical reasoning process should always begin with the following questions:^{7,17,22,37,60-62}

- (a) Is the vaccination schedule incomplete? Are additional doses of the same vaccine still needed?
- (b) What is the risk–benefit ratio of continuing the vaccination schedule (i.e. weighed against the risk of disease)?

The workup should begin with a thorough clinical history, with particular attention to previous adverse drug reactions or reactions following immunization. Aspects important to assess include the extent of the reaction (local vs. systemic), the time to onset of the reaction (immediate vs. nonimmediate/delayed), and the need for rescue medication (H1 receptor antagonist, epinephrine). The manufacturer and batch of the vaccine suspected of causing the reaction must be ascertained and recorded. The vaccine package insert or prescribing information should be checked for components such as adjuvants

Table 3
COVID-19 vaccines and hypersensitivity reactions

Vaccine (manufacturer)	Legal status (as of January 2021)	Vaccine type	Excipients	Hypersensitivity reactions
CoronaVac (Sinovac, China)	EUA for use in China (essential workers and high-risk groups), Turkey Pending: Indonesia	Inactivated vaccine (formalin with alum adjuvant)	Aluminum hydroxide, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride	No anaphylaxis events reported during Phase 3 trials (33,620 participants)
Convidicea Ad5-nCoV (CanSino Biologics, Beijing Inst. Biotech., NPO Petrovax)	EUA in China (limited to military use only) Pending: Mexico	Recombinant adenovirus type 5 vector against spike RBD protein	N/A	No anaphylaxis events reported during Phase 3 trials (40,000 participants)
BBIBP-CorV (Sinopharm, Beijing Institute & Wuhan Inst. of Biological Products)	Full authorization for use in China, EUA in Bahrain, Egypt, UAE.	Inactivated SARS-CoV-2 (vero cells) + aluminum hydroxide adjuvant	Aluminum hydroxide, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide, sodium bicarbonate, M199	No anaphylaxis events reported during Phase 3 trials (48,000 participants)
Pfizer-BioNTech BNT162b2	EUA in Argentina, Bahrain, Canada, Chile, Costa Rica, Ecuador, EU, Israel, Jordan, Kuwait, Mexico, Oman, Panama, Saudi Arabia, Singapore, Switzerland, UK, USA, WHO. Pending: Australia, India, Japan	mRNA-based vaccine encoding the viral spike (S) glycoprotein	(4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis (2-hexyldecanoate)] (ALC-0315), 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide (ALC-0159), 1,2-Distearoyl-sn-glycero-3-phosphocholine cholesterol, potassium chloride, potassium dihydrogen phosphate, sodium chloride, disodium hydrogen phosphate dihydrate, sucrose, water for injection	No anaphylaxis events attributed to vaccine reported in clinical trials (~22,000 participants randomized to active dosing). Approximate incidence of anaphylaxis: 1:100,000 with routine use
Moderna mRNA-1273	EUA in Canada, EU, Israel, Switzerland, UK, USA	mRNA-based vaccine encoding the pre-fusion stabilized spike (S) glycoprotein	Lipids (SM-102, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 [PEG2000-DMG], cholesterol, and 1,2-distearoyl-snglycero-3-phosphocholine [DSPC]), tromethamine, tromethamine hydrochloride, acetic acid, sodium acetate, and sucrose	No acute anaphylaxis reactions reported in clinical trials (~15,000 participants randomized to active dosing)

Table 3 (continued)

COVID-19 vaccines and hypersensitivity reactions

Vaccine (manufacturer)	Legal status (as of January 2021)	Vaccine type	Excipients	Hypersensitivity reactions
ChAdOx1 (Oxford/AstraZeneca; Covishield in India)	EUA in Argentina, Dominican Republic, El Salvador, EU, India, Mexico, Morocco, UK. Pending: Australia, Canada	Replication-deficient viral vector vaccine (adenovirus from chimpanzees)	L-Histidine, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80, ethanol, sucrose, sodium chloride, disodium edetate dihydrate, water for injection	No anaphylaxis events reported in clinical trials (~12,000 participants randomized to active dosing)
Covaxin (BBV152) (Bharat Biotech, India)	EUA in India	Inactivated vaccine	N/A	No events reported in Phase 1 studies (n = 300)
Sputnik V (Gamaleya Research Institute)	Russia, Palestine	Non-replicating, two-component vector (adenovirus) against spike (S) glycoprotein	Tris (hydroxymethyl) aminomethane, sodium chloride, sucrose, magnesium chloride hexahydrate, sodium EDTA, polysorbate 80, ethanol, water for injection	No events reported in Phase 1/2 studies (n = 76)
EpiVacCorona (Federal Budgetary Research Institution State Research Center, Russia)	Regulatory approval granted in Russia on basis of Phase 1/2 studies	Peptide vaccine with alum adjuvant	Aluminum hydroxide, potassium dihydrogen phosphate, potassium chloride, sodium hydrogen phosphate dodecahydrate, sodium chloride, water for injection	Unknown

Adapted from Turner PJ et al.⁵⁵.

(aluminium), preservatives (formaldehyde, thiomersal, 2-phenoxyethanol), stabilizers (lactose, gelatin), antibiotics, latex, and culture media (egg, yeast).^{61,62} If the history is consistent with a hypersensitivity reaction to the vaccine, further workup should be directed by the hypersensitivity mechanism involved (Figure 1).

Skin tests with vaccines or their components, when recommended, should only be performed by a trained team in an appropriate environment, under the supervision of an allergist. All skin tests must be interpreted carefully, with both positive and negative

controls. Positive results should be considered as indicative of sensitization, but not as proof of allergy, since false positives may occur in patients with no relevant clinical manifestations or may simply be due to the irritating effects of the antigen used for the test.

In patients who had an immediate hypersensitivity reaction, immediate-reading (skin prick or intradermal) tests with the vaccine and its components should be performed; if sensitization to a particular vaccine is demonstrated, vaccination can still be carried out in graded doses.^{61,62} Premedication with antihistamines

after skin testing and before vaccination with graded doses is controversial, and is not recommended before an initial or booster dose.

A number of approaches to vaccine skin testing have been suggested, but current guidelines recommend that prick testing be initiated with undiluted vaccine (1:1) unless the patient has a history of severe anaphylaxis. In such cases, it is appropriate to dilute the vaccine to 1:10 or even 1:100, which are considered non-irritating concentrations.⁶³

If the skin prick test is negative, an intradermal test with the vaccine at 1:100 dilution should then be performed.^{7,60,62} Intradermal testing with undiluted vaccine is discouraged due to the high rate of irritation. False-positive results due to irritative reactions are more frequent with pure or 1:10 vaccine, especially with the influenza, MMR, and varicella vaccines. At 1:100 dilutions, irritability reactions are far less common: 5% for DT and DTaP and 15% for influenza.^{7,22,55,63,64} Whenever possible, the test should be performed with the exact same vaccine (i.e. same manufacturer) suspected to have elicited the reaction.

If a hypersensitivity reaction is confirmed, another option to continue immunization is to switch to another vaccine for the same antigen but that does not contain the allergen putatively implicated in the reaction.^{7,22,55,63} Local non-allergic reactions, such as redness, pain, and edema, do not contraindicate administration of subsequent vaccine doses; nor does fever.^{7,22,55,63}

Immunization should not be delayed, and it is incumbent upon the allergist to carry out appropriate assessment and case management. With the decline in vaccination coverage observed in Brazil since the 1990s, the proportion of the population susceptible to infectious diseases – often highly communicable ones with high morbidity and mortality rates – has increased. In 2020, 8,000 cases of measles had already been confirmed in Brazil as of October. A specialist physician (allergist/immunologist) must be engaged to elucidate the case, together with the facility where vaccination took place or, in Brazil, the local referral center for special immunobiologicals (*Centro de Referência em Imunobiológicos Especiais*, CRIE).⁶⁵

Once again, it bears stressing that severe reactions to vaccines are rare events. Nonetheless, all sites where vaccines are administered must have the necessary supplies on hand for management of immediate hypersensitivity reactions: epinephrine,

antihistamines, corticosteroids, beta-2 agonists, and a supplemental oxygen source.⁷

Diagnostic workup

For assessment of a possible anaphylactic reaction to a vaccine, whenever possible, serum tryptase should be measured within 2 hours of the reaction and a baseline serum tryptase measurement should be obtained 48 hours after the reaction.²²

If a severe allergic reaction to a vaccine is suspected, identification of the causative allergen is paramount, as this may allow the administration of another formulation of the same vaccine, as well as allow the patient to avoid other products that contain the same allergen in future. If additional doses of the vaccine should be administered, a skin test with the vaccine and/or its components should be performed. This process can be relatively simple if a single-antigen vaccine was administered, or more complicated if simultaneous or combined vaccination (e.g. MMR) was performed, as is common in pediatric practice.⁷

Investigation of potential allergens present in the vaccine can be performed *in vitro* and/or *in vivo*, depending on the availability of allergen extracts. Specific IgE tests for most antimicrobial components are not commercially available, and at any rate are often useless in predicting allergic reactions to vaccines. For some other components (e.g. ovalbumin and gelatin), the ability of tests to predict a vaccine reaction is very low.²² Nevertheless, if the vaccine suspected to have caused the reaction contains specific components known to be potentially allergenic (e.g. egg, gelatin, latex or yeast), allergen screening should be conducted for these components. A simple gelatin prick test can be prepared by dissolving a teaspoon of powdered gelatin in 5 mL of normal saline solution.

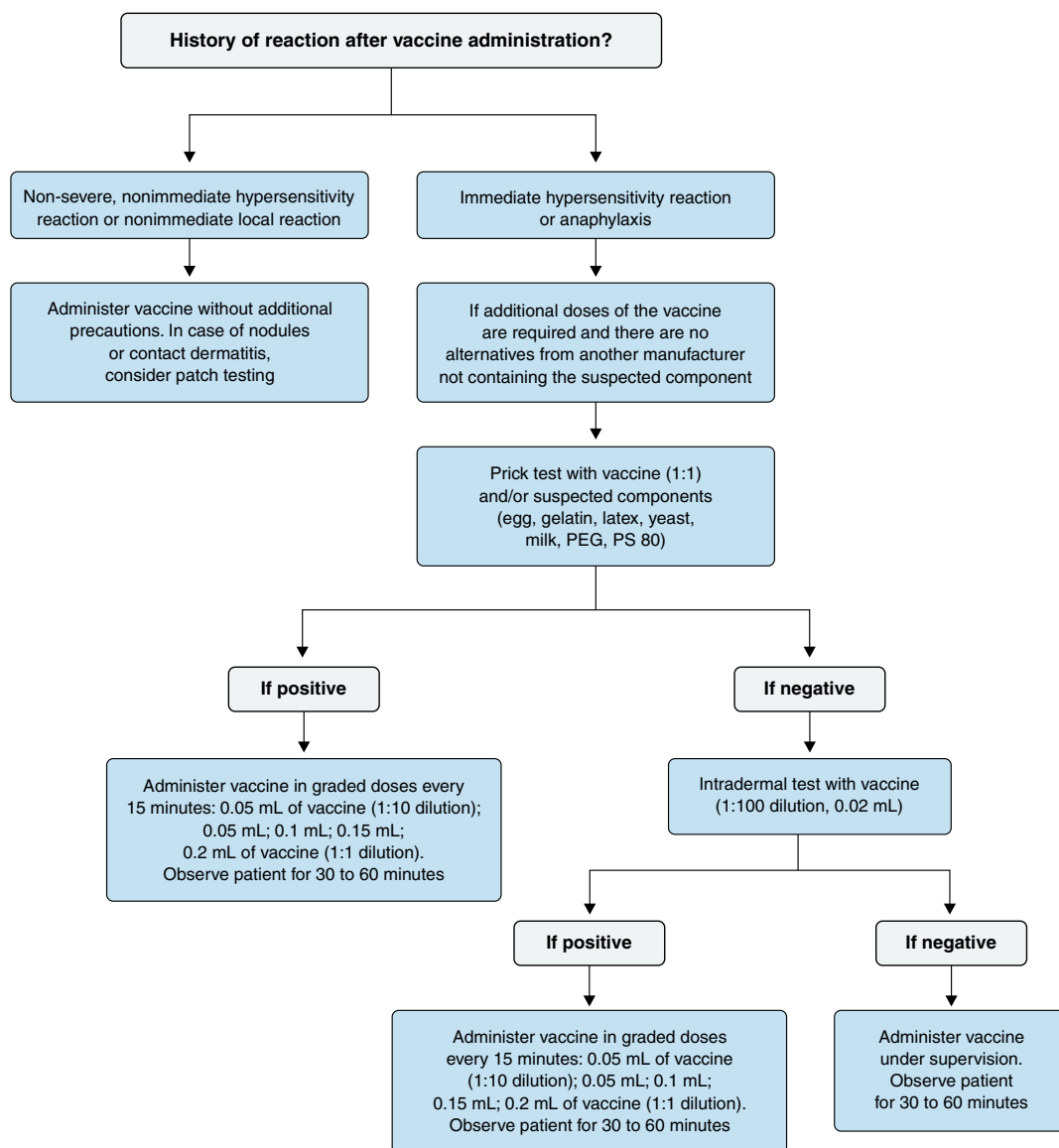
Nonimmediate local reactions, such as contact dermatitis, subcutaneous nodules, or type IV hypersensitivity reactions to preservatives, aluminum, and antibiotics, can be evaluated through patch testing. Although patch testing is not essential for therapeutic decision-making, it can help in choosing alternative vaccines where these are available.⁷

Allergen-specific *in vitro* IgE tests for egg, gelatin, latex, and yeast are available at most commercial laboratories. Examples of skin and serology tests that would be appropriate in evaluating suspected reactions to specific vaccines are given in Table 4.⁷

Anaphylaxis and its management

Anaphylaxis is a rapidly progressive, life-threatening systemic hypersensitivity reaction involving at least two organ systems: *skin and mucosa* (most frequently

involved, in >90% of cases, with erythema, pruritus, hives, and angioedema); *respiratory tract* (involved in 40% to 70% of cases, with hoarseness, cough, stridor, wheezing, dyspnea, chest tightness, asphyxia, death); *digestive tract* (affected in 30% of cases, with



Read at 15 min, consider positive if wheal = 3 mm. Positive (histamine) and negative (saline) controls. If skin test with vaccine negative: consider intradermal (ID) testing.

Consider test positive if the final wheal is twice as large as the initial wheal (ID). Obtaining written informed consent from the patient or guardian before the procedure is mandatory, as is a detailed explanation of the risks and benefits of desensitization.

Figure 1

Algorithm for investigation of hypersensitivity reactions to vaccines

nausea, vomiting, abdominal cramps, and diarrhea); *cardiovascular system* (tachycardia, hypotension, dizziness, syncope or presyncope, shock – in 10% of cases – and death); and/or *nervous system* (syncope, convulsions, coma). The estimated incidence of anaphylaxis is 10 to 20 cases per 100,000 person-years, with a documented upward trend in recent decades. With vaccines, the incidence is even lower, at approximately one case per million doses. Prompt treatment is critical for recovery, and delay in administration of epinephrine has been identified as a factor in unfavorable outcomes. It cannot be overstated that the first-line drug of choice in anaphylaxis is epinephrine IM, only later followed by antihistamines and corticosteroids.³⁷

Other factors that may have a negative impact on the outcome of anaphylaxis are the use of inadequate doses or routes of administration for epinephrine (e.g. subcutaneous) and the concomitant use of other drugs, such as beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, monoamine oxidase (MAO) inhibitors, and antidepressants. After the acute episode, a second late phase may occur, with symptoms recurring after 6 to 12 hours. Therefore, patients must remain under close observation for at least 12 hours after an initial anaphylaxis event.³⁷

Reporting

In 1992, to comply with World Health Organization (WHO) recommendations, the Brazilian Ministry of Health National Immunization Program (PNI/MS) began developing the Nationwide Surveillance System for Adverse Events Following Immunization (*Sistema Nacional de Vigilância de Eventos Adversos Pós-Vacinais*, SNVEAPV). Its objectives include standardizing recognition of suspected ESAVI and their management, providing in-depth knowledge on the nature of these events, and identifying potential breakdowns in transport, storage, handling, or administration (immunization errors) that result in adverse events. A careful analysis of the causality of the adverse event and its potential relationship with the administered product is conducted, and the resulting information – including incidence and severity of the observed reactions – is disseminated.⁵

Recognizing the importance of surveillance for this type of adverse event, the MoH Department of Health Surveillance (*Secretaria de Vigilância em Saúde*, SVS) has made all EAPVs notifiable events (Ordinance No. 33/SVS/MS of 2005, subsequently revoked by Ordinance No. 1.271/SVS/MS of 2014).⁵

Table 4

Examples of testing used to screen for sensitization to specific vaccines or components

Vaccine	Skin testing	Serum specific IgE testing
DTaP, Td, Tdap	DTaP, Td, Tdap, tetanus toxoid, gelatin, milk	Gelatin, milk, latex
Hepatitis B	Hepatitis B, yeast	Yeast, gelatin, latex
Influenza	Influenza, egg, gelatin	Egg, gelatin, latex
MMR	MMR, measles, mumps, rubella, gelatin	Gelatin, latex
Varicella or Zoster	Varicella or Zoster, gelatin	Gelatin, latex
Yellow fever	Yellow fever, egg, gelatin	Gelatin, latex

To wit:

- Any adverse effect caused by immunization must be reported to the National Health Surveillance Agency (ANVISA) through the Health Surveillance Notification System (NOTIVISA). The time elapsed between vaccination and onset of symptoms should be considered.
- Local reactions such as abscess, cellulitis or nodulation at the site of administration; induration or swelling near or at the site of administration; granuloma; Arthus reaction; pain at the site of administration, at the time of administration or shortly after receiving the vaccine, must be reported.
- Systemic reactions such as anaphylaxis, as well as anaphylactoid reactions and anaphylactic shock, must also be reported. As must: inconsolable crying, lasting 3 hours or more, up to 48 hours after vaccine application; seizures, encephalitis, myelitis, acute disseminated encephalomyelitis; encephalopathy; hypotonic-hyporesponsive episode; fever; intestinal intussusception; aseptic meningitis; narcolepsy; optic neuritis; facial palsy; inflammatory polyradiculoneuropathies, such as the Guillain-Barré Syndrome; vasovagal syncope; Complex Regional Pain Syndrome; Early Systemic Reaction Syndrome; thrombocytopenia or low platelet count.
- Deaths temporally associated with adverse events following immunization, such as Sudden Unexplained Death in Childhood, Sudden Infant Death Syndrome, and all deaths with ill-defined causes, must also be reported. In these cases, reporting must be done in coordination with the medical examiner's office or coroner's office. Where available, it is recommended that a postmortem examination be performed within the first 72 hours, before tissue damage sets in which could hinder diagnosis.

Conclusions

It is part of health care providers' duty to assess the vaccination status of their patients and caregivers and formally recommend those vaccines indicated in the corresponding immunization schedule, considering age, special conditions (allergies and immunodeficiencies), and possible contraindications and precautions. In addition to recommending the necessary vaccinations, the allergist/immunologist

must be prepared to address any vaccine-related adverse events, accurately establishing a diagnosis and providing the appropriate guidance and management.

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Understanding before incorporating: a portrait of milk allergy programs implemented in Brazil

Conhecer antes de incorporar: um retrato dos programas para alergia ao leite implementados no Brasil

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ABSTRACT

This paper aims to characterize the assistance offered to children with cow's milk allergy in public programs and challenges to their implementation, specifically assessing the pre-incorporation phase in the Brazilian Unified Health System of 3 formulas for infants with milk allergy. This exploratory, cross-sectional study with a quantitative approach assessed 21 programs/services from all regions of the country. The main motivation for the creation of these programs was judicialization (80.9%), and these special formulas were provided for children up to 2 years of age. The main challenges to creating and implementing these programs were a lack of human and financial resources, no counterpart federal program, no unified protocol for diagnosis (oral provocation test), and the selection of formula types. The most common strategy for reducing costs was updating norms and protocols (61.9%), which did not differ significantly between state and municipal programs. This study presents an unprecedented and detailed evaluation of the programs, raising discussion about decision-making, the rational use of public resources, better care for children, and means of strengthening of the national health system.

Keywords: Unified Health System, program evaluation, continuity of patient care, milk hypersensitivity, health manager.

RESUMO

Este trabalho objetiva caracterizar a assistência ofertada às crianças com alergia ao leite em programas públicos, e os desafios enfrentados na sua implantação, no contexto da pré-incorporação no Sistema Único de Saúde, de três fórmulas infantis para alergia ao leite. Estudo exploratório, transversal e abordagem quantitativa. Foram avaliados 21 programas/serviços de todas as regiões brasileiras. O principal indutor da criação destes programas foi a judicialização (80,9%), e o fornecimento destas fórmulas especiais foi realizado para crianças com até 2 anos de idade. Os principais desafios para a criação e execução destes programas foram a falta de recursos humanos e financeiros, a falta da contrapartida da União, protocolo unificado para o diagnóstico (Teste de Provocação Oral), e a escolha dos tipos das fórmulas. A estratégia mais adotada para redução dos custos foi a adequação das normas e protocolos (61,9%). Não houve diferença significativa entre os programas estaduais e municipais. Este estudo apresenta uma avaliação inédita e detalhada sobre os programas, trazendo discussões que corroboram a tomada de decisões, o uso racional de recursos públicos, a melhor assistência às crianças e o fortalecimento do sistema de saúde nacional.

Descritores: Sistema Único de Saúde, avaliação de programas e projetos de saúde, continuidade da assistência ao paciente, hipersensibilidade a leite, gestor de saúde.

Introduction

Allergic diseases are an important cause of morbidity worldwide and are a considerable burden to health systems in emerging and developed economies.¹ Cow's milk protein allergy (CMPA) is

an immune response to cow's milk protein fractions, especially α -lactalbumin, β -lactoglobulin, and casein.² This food allergy can compromise several organ systems, although the gastrointestinal, skin, and

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respiratory systems are most affected.³ CMPA is the most common food allergy in children, especially newborns.^{4,5} The severity of CMPA reduces over time in most children⁶, given that certain factors contribute to earlier cow's milk tolerance, such as CMPA not mediated by immunoglobulin E, and treatment with hydrolyzed casein + probiotics (strains of *Lactobacillus rhamnosus*). Factors such as cesarean delivery and breastfeeding < 3 days contribute to late tolerance.⁵ CMPA diagnosis is complex, based on several stages.⁷

For the mothers of children who have a reaction to breast milk proteins, the current Brazilian Consensus on Food Allergy⁶ recommends following an exclusion diet and monitoring the infant's symptoms. For formula-fed infants, the recommendation is to exclude CMP-based formulas, replacing them with extensively hydrolyzed formulas (EHF) based on cow's milk protein. If there is no clinical improvement after 2 weeks, these should be substituted with amino acid-based formulas (AAF).^{6,8,9} If the child shows improvement, an oral provocation test (OPT) must be performed. Positive OPT results indicate that the exclusion diet must be continued with the same formula for 6 to 12 months.⁶ In Brazil, no national level surveys have been conducted on the prevalence of CMPA.^{10,11}

According to Patton¹², "Systematic collection of information about the activities, characteristics and the results of these programs to pass judgment on them, improve its effectiveness and clarify decisions related to new software". This study was based on this definition; studies evaluating programs that provide special infant formulas are scarce, being generally related to the epidemiological and clinical characterization of patients¹³, histories of the creation of such programs¹⁴, or reports on interventions to encourage breastfeeding.¹⁵ This is the first nationwide study to describe and evaluate programs/services, reflecting their differences and specificities.

In 2012, discussion began about incorporating these special formulas for children with CMPA into the Brazilian Unified Health System (UHS) due to increased demand, the growing judicialization of states and municipalities and, a lack of initiatives at the federal level, proposals have been made to incorporate 3 types of formulas for children with CMPA: (1) soy-based (SBF), (2) EHF, and (3) AAF.¹⁰ In 2014, this measure was unanimously approved but not implemented until fiscal responsibility could be determined at federal, state, and municipal levels.

Since no conclusions were reached, the proposal was resubmitted in 2017, but was not implemented on a national level. The programs that currently exist are state and municipal initiatives.

According to the National Food and Nutrition Policy¹⁶, food and nutrition are basic conditions for health promotion and protection, allowing unrestricted growth and development with dignity and quality of life. However, this does not extend to special health conditions, such as food allergies. This underscores the need for discussion about improving care for children with food allergies and their families to guarantee health, citizenship, and quality of life.

Due to the relevance of the topic, the functioning, routines, and challenges directly involved in current programs and/or services that provide infant formula, as well as national-level programs still in the implementation phase, must be better understood before incorporating this new technology in the UHS to ensure greater equity and coverage.

Methods

This exploratory, quantitative, cross-sectional study identified municipalities and states with programs/services to assist children with CMPA, either through direct consultation with the Ministry of Health, the National Council of Health Departments, or the National Council of Municipal Health Departments (34 municipal and 5 state programs identified) or through an Internet search using the terms "program", "allergy" and "cow's milk" (16 municipal and 4 state programs identified).

After this step, the protocols and regulations of these programs were studied, and those that only provided formula due to court decision were excluded. Thus, a total of 35 programs were considered eligible (26 municipal and 9 state programs). Since the Sergipe State Department of Health and the Aracaju Municipal Health Department programs were formed through an agreement with the State Attorney General, we considered them to be a single program. The same occurred with the Vitória municipal program and the Espírito Santo state program. Thus, the total sample was 25 municipal and 9 state programs (Figures 1 and 2).

To evaluate the existing programs, the states and municipalities were contacted through official institutional channels (eg, website "Contact Us" links, telephone numbers, or social media channels). A

database was produced with this information to speed up communication. However, much of this information was out of date, which led to new searches and contact with other health departments in an effort to reach the relevant secretaries of health and locate the sectors responsible for the program.

Data were collected through a semi-structured interview by telephone and/or videoconference (Microsoft Teams or Google Meet) with the program coordinators appointed by the respective health departments. Data pending at the time of the interview were requested later via e-mail. The script of the interview was based on the program's protocols and a literature review, being divided into the following 5 sections (28 total questions):

- *Institutionalization*: program level (municipal or state), official program name, starting year, motivation, department responsible for managing the program administratively and financially, partnerships, and communication with other health departments;
- *Epidemiological data, formula types, and the direct costs of each program*: the number of children assisted and the cost of formula acquisition in the last five years (2015 to 2019), the provision of formula to children > 2 years of age, the most used formulas in children < and > 6 months of age;
- *Team and routines*: the professionals who directly assist children with CMPA, the minimum and maximum time from registration until the first formula is received, periodicity of monitoring by a professional, testing within the program, factors considered in CMPA diagnosis, oral provocation testing, and the conditions for performing OPT;
- *Breastfeeding*: guidance about the importance of breastfeeding, providing educational material about the exclusion diet, and institutions or initiatives to support breastfeeding in the city/state;
- *Managerial view*: the main difficulties in implementing and executing the program, suggestions for resolving reported difficulties, and cost reduction strategies.

The interviews were preceded by telephone and/or institutional e-mail contact. The provided services (diagnosis, access, and monitoring) were characterized using data officially available through the health departments. The interviews were conducted between June 2020 and June 2022.

The data were organized in tables and descriptive

statistical analysis was performed, including calculations of simple and relative frequencies for qualitative variables, and measures of central tendency and dispersion for quantitative variables. In the inferential analysis, the chi-square test of homogeneity was used to determine whether a random variable behaved similarly (homogeneously) in several categories of the same variable. The significance level was set at 5%, and IBM SPSS Statistics 20.0 and R 3.6.0 were used for the analyses.

The project was approved by the University of Brasília Faculty of Health Sciences Research Ethics Committee (29583520.4.0000.0030, opinion 3.984.775), the Curitiba Municipal Health Department Ethics Committee (29583520.4.3001.0101, opinion 4.852.974), the Belém Permanent Education Nucleus (authorized on April 28, 2021), the Espírito Santo Institute of Education, Research, and Innovation in Health's Research Sector (authorized on August 20, 2020), and the Maringá Permanent Commission for Process Evaluation (authorized on July 2, 2021).

Results

The study included 21 programs/services. The 15 municipal programs were in Afogados da Ingazeira (PE), Belém (PA), Belo Horizonte (MG), Blumenau (SC), Campos Altos (MG), Caruaru (PE), Contagem (MG), Curitiba (PR), Dourados (MS), Florianópolis (SC), Lucas do Rio Verde (MT), Maringá (PR), Rio Verde (GO), São José (SC), and Várzea de Palma (MG). The 6 state programs were in Ceará, the Federal District, Espírito Santo, Maranhão, Rio Grande do Norte, and Sergipe).

The municipal program coordinators in Almirante Tamandaré (PR), Camaçari (BA), Jaboatão dos Guararapes (PE), Jaguariúna (SP), Lupionópolis (PR), São Lourenço (MG), São Sebastião do Paraíso (MG), Sete Lagoas (MG) and Uruana (GO), and the state coordinators of São Paulo and Mato Grosso do Sul did not respond after numerous attempts via telephone and e-mail. The state coordinator of Rio Grande do Sul replied that it was not possible to participate in the study.

Most of these programs were embedded in other programs that provide enteral formulas and other special dietary products (state: the Federal District, Espírito Santo, and Sergipe; municipal: Campos Altos, Caruaru, Contagem, Blumenau, Curitiba, and Rio Verde), food allergy programs (Afogados da

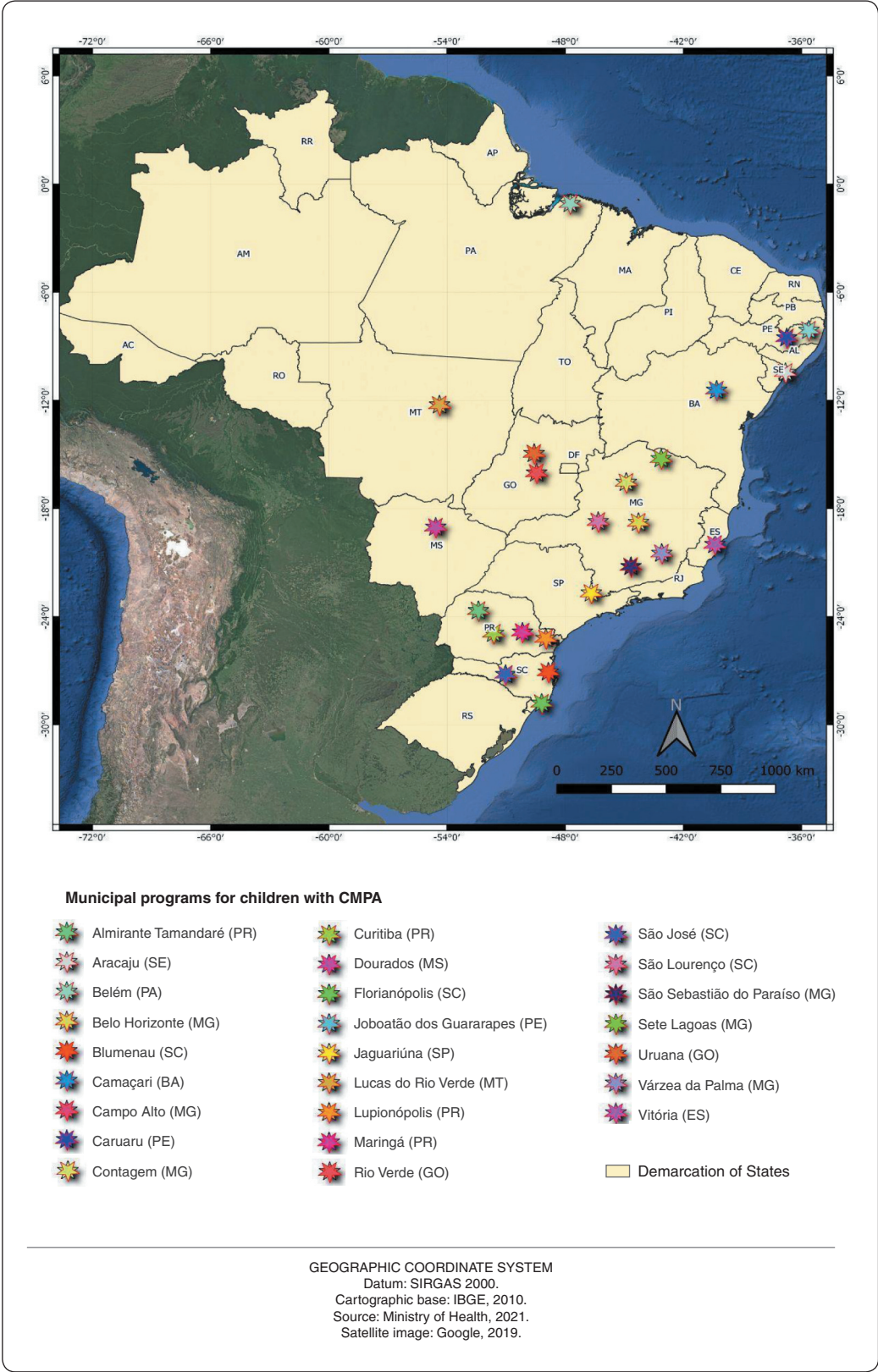


Figure 1
Geographical distribution of Brazilian municipalities with programs that provide formula for children with cow's milk protein allergy (CMPA), Brazil, 2022

Ingazeira, Belém), or programs for special needs children (Dourados, Florianópolis, Maringá, São José, Várzea de Palma); programs exclusively for CMPA (state: Ceará, Maranhão, and Rio Grande do Norte; municipal: Belo Horizonte), or as a specialized

component of a municipal program (Lucas do Rio Verde).

Judicial action was the main motive behind the creation of these programs (80.9%) (Table 1). All programs were linked, administratively and financially,

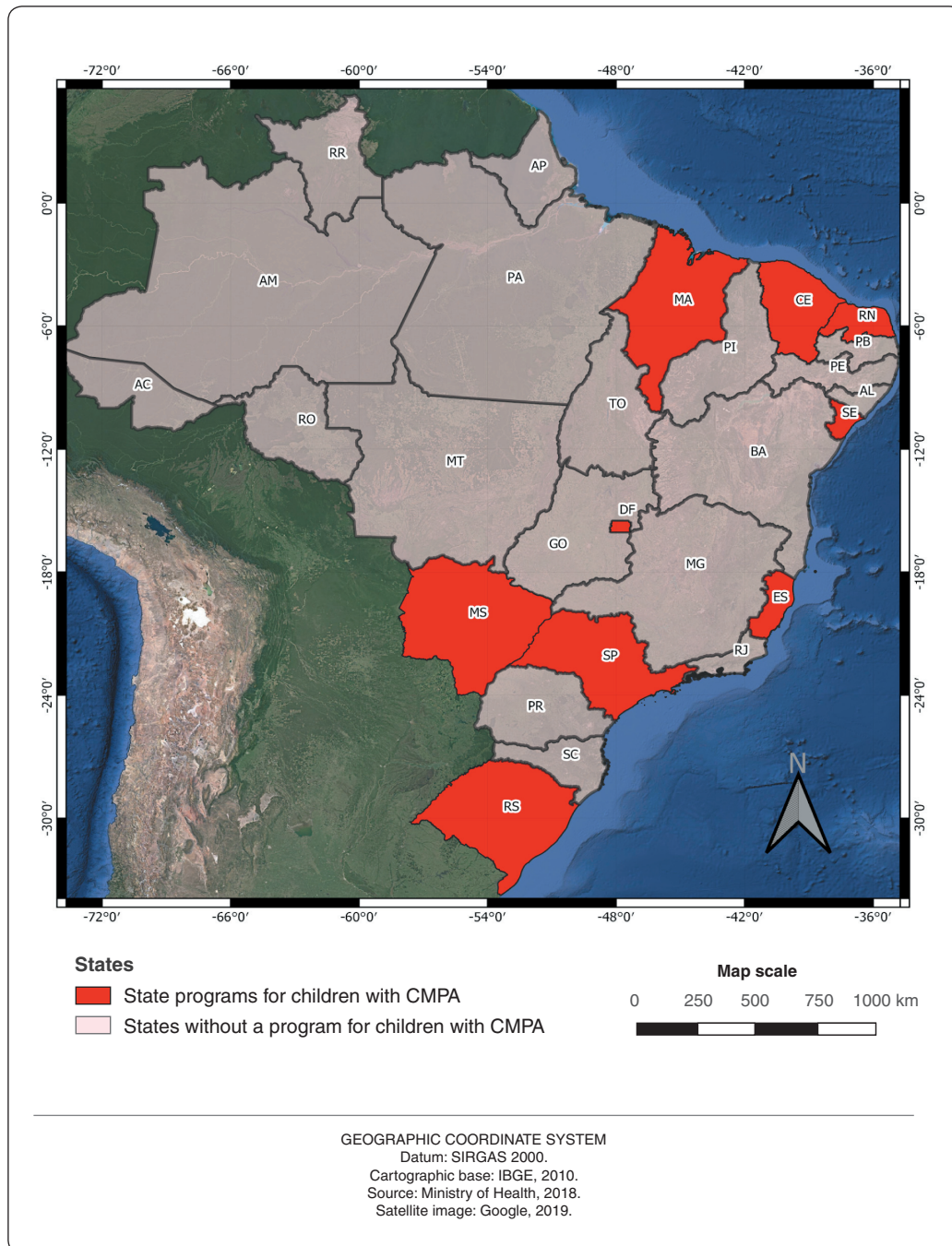


Figure 2

Map layout of Brazilian states with programs that provide formula for children with cow's milk protein allergy (CMPA), Brazil, 2022

to municipal or state health departments, and 80.9% had no partnership or planned communication with other health departments.

The programs in Blumenau, Curitiba, and São José communicate with the departments of education and social service, the department food and nutrition security (only Curitiba), and the municipal social service department (Campos Altos)(Table 1). Partnership, in this context, refers to pre-established formal relationships within the program, in addition to informal communication not established in the program regulations.

The secretary of education was contacted regarding assisted children enrolled in municipal daycare centers to guarantee safe meals without allergens and an adequate supply of formula according to the time spent in daycare. The department of social service was contacted to verify sufficient environmental hygiene to safely prepare the formula, the family's social conditions, and the social reception of the program, including guidance about other programs. The Curitiba program reported communication with the municipal Food and Nutritional Security Department, which instructs families in vulnerable situations to register with the "Family Warehouse" Program to gain access to basic products below market prices.

In most programs (Table 2) special formulas are provided until the children reach 2 years of age, although 9 programs (state: Maranhão, Rio Grande do Norte, and Sergipe; municipal: Afogados da Ingazeira, Belém, Curitiba, Florianópolis, Lucas do Rio Verde, and Maringá) allow extensions based on evaluation by specialists and/or the program's internal committees. The most widely available formulas for children < and > 6 months of age were lactose-free EHF and AAF; SBF was also widely used for children > 6 months of age (Table 2).

Table 3 shows the number of children assisted by each program in the last 5 years (2015 - 2019) and the direct costs of special formulas. However, inferences could not be made about the relationship between follow-up routines, program format, and cost per child, since most of the programs did not keep such data. However, if only the programs with complete data are considered (Espírito Santo, Maranhão, Blumenau, and Curitiba), the average annual cost per child varied from BRL 1663.00 (Curitiba) to BRL 2081.00 (Espírito Santo), which is close to the expected value. The cost in Blumenau (BRL 6400.00) was 3 times that of Espírito Santo, while that of Maranhão (BRL

17,640.60) was 8 times that of Espírito Santo. Thus, the second major question arose: what is the cause of the large variations?

Some factors that might explain the cost discrepancy between Maranhão's Special Milk Program and the other programs include providing formula for children up to 36 months of age, inconsistencies regarding the use of soy-based formula (SBF) in children < 1 year of age (SBF and rice-based infant formula are not recommended for children < 1 year of age), the lack of an allergologist or pediatric allergist on the program's evaluation committee, in addition to logistics and transportation costs. The children are monitored by pediatric gastroenterologists and nutritionists linked to the UHS, and OPT is performed every 6 months at home; however, in cases of immunoglobulin E-mediated CMPA, milk must be reintroduced in conjunction with OPT in a controlled environment. The number of cans provided per month is limited according to age: 8 per month for children ≤ 12 months old, 6 per month for children ≤ 24 months old, and 4 per month for children ≤ 36 months of age (Government of Maranhão, n.d.) .

SBF is a viable alternative due to its lower cost and the fact that it can be given to children with immunoglobulin E-mediated CMPA beginning at 6 months of age.⁶ The specialized municipal program in Lucas do Rio Verde does not provide formula for children < 6 months of age, while for those > 6 months of age it only provides only SBF, which can explain the program's low annual cost (BRL 204.17) per child. However, the Ceará program does not provide SBF, reasoning that it would lead to greater permanence in the program, which would increase costs, given that there are similar products that could be incorporated in the program due to new court decisions.

The Maringá program was the only one to mention, in addition to the standard formulas for CMPA (AAF, EHF and SBF), a rice-based formula, whose use has not been approved by National Commission for Incorporating Technologies in the Unified Health System. Since only one manufacturer produces this formula, competition is impossible, which could increase program costs. However, because this type of formula was only released in 2021, its effects have not yet been observed.

The programs in the states of Ceará, Espírito Santo, Maranhão and in the city of Belém (PA) also provide AAF to children > 1 year of age (1 to 10 years) as an additional alternative. This, too, is also produced by a single manufacturer, with no equivalent product

available on the market.

Programs that provide more AAF have higher costs than those that provide more EHF and SBF, although all 3 alternatives are necessary. Factors such as distribution logistics and administrative purchasing processes also contribute to these differences. In 2022, the National Commission for Incorporating

Technologies in the Unified Health System¹¹ reported that the lowest price for each product was BRL 15.12 for SBF, BRL 20.33 for EHF, and BRL 36.37 for AAF, although these values are now out of date. Since the programs did not keep records on the amounts spent on each type of formula during this period, the totals cannot be estimated.

Most programs provide guidance on the importance

Table 1

Programs and services that provide formula for children with cow's milk protein allergy (CMPA), Brazil, 2022

	N°	%
Program level		
State	6	28.6
Municipal	15	71.4
Program start date		
< 2000	1	4.8
2001-2010	8	38.1
2011-2020	12	57.1
Justifications for creating the programa		
Increased demand	8	38.1
Absence of similar initiatives at the federal and state levels	2	9.5
Suppressed demand	2	9.5
Lack of patient follow-up	4	19
Judicialization or other legal issues	17	80.9
To organize the flow of formula provision	2	9.5
Rational use of public resources	5	23.8
Department to which the program and/or service is linked		
Health	21	100
Partnerships and/or communication with other departments		
No	17	80.9
Yes	4	19.1
Social assistance	4	
Education	3	
Food and nutrition security	1	

^a Food and nutrition security: p-value < 0.001.

of breastfeeding and an exclusion diet (Table 2), which should include instructions about restricting milk and milk derivatives, guidance about reading labels, technical terms indicating the presence of milk and its protein fractions, as well as about foods that the mother can consume to avoid unnecessary restrictions. A total of 71.4% of the programs reported providing educational materials about the exclusion

diet. Most program managers (76.2%) mentioned initiatives to promote and protect breastfeeding in their territories.

The most common type of professional involved in the follow-up of children with CMPA was nutritionists (85.7% of the programs; n = 18) (Table 4), except in Espírito Santo, where mandatory follow-up was only performed by professional assistants, Florianópolis,

Table 2

Formula types and maternal nutrition materials provided in programs for children with cow's milk protein allergy (CMPA), Brazil, 2022

	Nº	%
Formula provided for children > 2 years of age?		
Yes	3	14.3
No	18	85.7
Formulas provided to children < 6 months of age		
AAF	18	85.7
RBF	1	4.8
EHF with lactose	14	66.7
EHF without lactose	19	90.5
FSO	1	4.8
Unavailable	1	4.8
Formula for children > 6 months of age		
AAF	19	90.5
AAF for children ≥ 1 year of age	4	19.1
RBF	1	4.8
EHF with lactose	14	66.7
EHF without lactose	19	90.5
SBF	18	85.7
Guidance on the importance of breastfeeding in CMPA and exclusion diet		
Yes	17	80.9
No	4	19.1
Receipt of educational material on diet of exclusion		
Yes	15	71.4
No	6	28.6
Presence of institutions/initiatives/incentive projects for breastfeeding		
Yes	16	76.2
No	5	23.8

AAF = amino acid-based formula, EHF = extensively hydrolyzed formula, SBF = soy-based formula, RBF = rice-based formula.

Table 3
Number of children and direct costs for programs that provide formulas for children with cow's milk protein allergies in the last 5 years, Brazil - 2022

Variables	Home Enteral Nutritional Therapy Program (DF)	State Food Allergy Program (SE)	Decree 054R Apr 28, 2010 (ES)	Municipal Program for Dispensing Special Infant Formulas, Florianópolis (SC)	Special Food Program, Blumenau (SC)	Nutritional Care Program for People with Special Food Needs, Curitiba (PR)	Food Allergy Program, Belém (PA)	Special Infant Formula Program/ Nutritional Care Program, Rio Verde (GO)	Special formula program for patients with CMPA (RN)	Nutrir, Program, Dourados (MS)	Infant Formula Program, Maringá – (PR)
2015	N° of children	395	NI	1211	33	617	NI	291 ^d	NI	NI	NI
BRL	NI	2,450,376.44	NI	201,834.68	201,834.68	1,009,657.22	NI	NI	NI	NI	NI
BRL/child	–	2,023.43	–	6,116.20	6,116.20	1,636.4	–	–	–	–	–
2016	N° of children	427	NI	1,636	38	629	385	266 ^d	NI	NI	NI
BRL	NI	3,683,029.46	NI	215,811.12	215,811.12	884,428.9	NI	NI	NI	NI	NI
BRL/child	–	2,251.24	–	5,679.24	5,679.24	1,406.09	–	–	–	–	–
2017	N° of children	280	NI	1,830	36	664	329	227	NI	NI	NI
BRL	2,172,222.48	NI	4,159,091.60	NI	204,753.54	1,389,074.04	NI	NI	NI	NI	NI
BRL/child	–	2,272.73	–	5,687.60	5,687.60	2,091.98	–	–	–	–	–
2018	N° of children	362	NI	1,958	33	812	352	223	NI	NI	NI
BRL	NI	4,287,135.05	NI	223,792.80	223,792.80	1,200,436.74	3,784,000.00	NI	NI	NI	NI
BRL/child	–	2,189.55	–	6,781.60	6,781.60	1,478.37	10,750.00	–	–	–	–
2019	N° of children	395	NI	1,791	43	773	398	150	NI	NI	175
BRL	3,689,853.44	NI	2,958,933.64	1,557,451.83	340,923.84	1,330,234.99	4,207,000.00	NI	NI	NI	931,86.00
BRL/child	9,341.4	–	1,652.11	1,106.93	7,928.46	1,720.87	10,570.35	–	–	–	5,324.91
Total	N° of children	675 ^a	–	8,426	183	3,495	750 ^c	1,157 ^d	NI	NI	175 ^e
BRL	5,862,075.92 ^a	–	17,538,566.19	1,557,451.83 ^b	1,187,115.98	5,813,831.89	7,991,000.00 ^c	NI	NI	NI	931,860.00 ^e
BRL/child	8,684.56 ^a	–	2,081.48	1,106.93 ^b	6,486.97	1,663.47	10,654.67 ^c	–	–	–	5,324.91 ^e

BRL = Brazilian Reals; CMPA: cow's milk protein allergy; NI = Not informed.

^a Considering the total number of children assisted by Home Enteral Nutritional Therapy Program and the costs in 2017 and 2019 (complete data); not all of the children had CMPA, but according to the manager, they were the majority.

^b Considering the total number of children assisted by the Municipal Program to Provide Special Infant Formulas (Florianópolis, SC) and the 2019 costs (complete data).

^c Considering the total number of children assisted by State Food Allergy Program (PA) and costs in 2018 and 2019 (complete data).

^d In 2015 and 2016 Rio Verde (GO) program provided formula to children with CMPA up to 3 years of age; as of 2017 the limit was reduced to 2 years of age.

^e Considering the total number of children assisted by the Maringá Special Infant Formula Program and costs in 2019 (complete data).

^f Considering the total number of children assisted by the specialized municipal program of Lucas do Rio Verde (MT) and the costs for 2017 and 2018 (complete data).

^g The CMPA clinics in Belo Horizonte (MG) provided formulas to children at least 1 year of age.

^h Considering the total number of children assisted at the CMAP clinic in Belo Horizonte (MG) and the costs for 2017, 2018 and 2019 (complete data).

Table 3 (continued)
Number of children and direct costs of programs that provide formula to children with cow's milk protein allergy in the last 5 years, Brazil, 2022

Variable	Milk Formula Program for Intolerant and Sensitive People, Afogados da Ingazeira (PE)	Municipal protocol for dispensing special industrialized nutritional formulas, Várzea de Palma (MG)	Protocol for Dispensing Food Formulas, Contagem (MG)	Enteral diet protocol of the municipal health system of Caruaru (PE)	Municipal Infant Distribution Program, Campos Altos (MG)	Specialized municipal component, Lucas do Rio Verde (MT)	Program for underprivileged children with food intolerance and/or allergy – Special Milk Program (MA)	São José children's program, São José (SC)	CMPA/ Specialized Medical Assistance Service (CE)	Municipal CMPA clinic, Belo Horizonte (MG)
2015	N° of children	NI	NI	NI	NI	NI	451	132	NI	16 ^g
BRL		NI	NI	NI	NI	NI	6,782,202.27	NI	NI	NI
BRL/child		–	–	–	–	–	15,038.14	–	–	–
2016	N° of children	NI	NI	NI	10	NI	474	99	NI	80 ^g
BRL		NI	NI	NI	NI	1,198.80	7,094,429.16	NI	NI	NI
BRL/child		–	–	–	–	–	14,967.15	–	–	–
2017	N° of children	NI	NI	NI	12	6	608	100	NI	153 ^g
BRL		NI	NI	NI	NI	959.00	9,719,704.98	NI	NI	682,800.94
BRL/child		–	–	–	–	159.83	15,986.36	–	–	4,462.75
2018	N° of children	NI	NI	NI	14	9	815	94	NI	226 ^g
BRL		NI	NI	NI	NI	2,103.53	15,389,690.54	NI	NI	579,896.64
BRL/child		–	–	–	–	233.73	18,883.06	–	–	2,565.91
2019	N° of children	NI	NI	NI	NI	3	750	107	NI	230 ^g
BRL		NI	NI	NI	NI	NI	15,664,564.07	NI	NI	499,282.56
BRL/child		–	–	–	–	–	20,886.09	–	–	2,170.79
Total	N° of children	NI	NI	NI	NI	15 ^f	3,098	532	NI	609 ^h
BRL		NI	NI	NI	NI	3,062.53 ^f	54,650,591.02	NI	NI	1,761,980.14 ^h
BRL/child		–	–	–	–	204.17 ^f	17,640.60	–	–	2,893.24 ^h

BRL = Brazilian Reals; CMPA: cow's milk protein allergy; NI = Not informed.

^a Considering the total number of children assisted by Home Enteral Nutritional Therapy Program and the costs in 2017 and 2019 (complete data); not all of the children had CMPA, but according to the manager, they were the majority.

^b Considering the total number of children assisted by the Municipal Program to Provide Special Infant Formulas (Florianópolis, SC) and the 2019 costs (complete data).

^c Considering the total number of children assisted by State Food Allergy Program (PA) and costs in 2018 and 2019 (complete data).

^d In 2015 and 2016 Rio Verde (GO) program provided formula to children with CMPA up to 3 years of age; as of 2017 the limit was reduced to 2 years of age.

^e Considering the total number of children assisted by the Maringá Special Infant Formula Program and costs in 2019 (complete data).

^f Considering the total number of children assisted by the specialized municipal program of Lucas do Rio Verde (MT) and the costs for 2017 and 2018 (complete data).

^g The CMPA clinics in Belo Horizonte (MG) provided formulas to children at least 1 year of age.

^h Considering the total number of children assisted at the CMAP clinic in Belo Horizonte (MG) and the costs for 2017, 2018 and 2019 (complete data).

where mandatory follow-up is performed by dietitians, and in Campos Altos, followed up is performed by pediatricians.

Although nutritionists are not part of mandatory follow-up, in Florianópolis they are a fundamental part of the multidisciplinary commission that evaluates cases which do not fit program criteria; they are also part of the Expanded Family Health Center team, which supports cases assisted in primary health care. In Espírito Santo, nutritionists are part of the technical expert group, and they are present in 95.2% of the evaluated programs in either assistance or management positions.

Specialist physicians (pediatric allergy specialists and pediatric gastroenterologists), pediatricians, and social workers were also well represented in follow-up routines. Follow-up periodicity varied according to specialty, averaging up to 3 months.

The clinical history (anamnesis) and evaluation of signs and symptoms were the main diagnostic methods for CMPA, followed by OPT (57.1%, $n = 12$) (Table 4). Program managers most often reported that the hospital was the best environment for performing OPT, with 80% considering it an adequate location if serious reactions (anaphylaxis) occur.

Program managers reported that the lack of human resources, especially professionals trained to organize work flows and routines, create protocols, and assist patients, was the main obstacle to implementing and executing these programs (Table 5). The lack of financial resources was also important, since no federal and few state initiatives exist to help these children, ie, municipalities were generally required to mobilize their own resources to maintain the programs.

Federal and state financial contributions, continuing education initiatives for health professionals, and the hiring of health professionals, mainly specialists, were cited as relevant actions for improving the management and scope of these programs (Table 5).

Developing and updating of standardized management protocols for CMPA patient care was reported as the most relevant way to reduce the financial costs of the programs (Table 5). Such protocols allow organized routines for patient care, identifying those who truly need formula, preventing inadequate preparation (errors in handling, dilution, and preparation [eg, with contaminated water]) and preventing fraud regarding these expensive formulas (eg, sale or distribution to third parties).

Discussion

Defining age limits is the *first challenge* of programs for children with CMPA, since, food source restrictions, especially situations beyond parental control, such as restaurants and day care centers, make introducing foods an even greater challenge. Children should be encouraged to shift from liquid to pasty foods and thus reduce formula intake, the previous main source of nutrition. This transition from a “safe” state, in which parents can safely prepare allergen-free food, to a new and more challenging scenario can lead to insecurity about the child’s growth, development, and health. Close continuous multidisciplinary follow-up helps reinforce the importance of a healthy, varied, and allergen-free diet, following national recommendations.¹⁷

A *second challenge* is determining which formulas to provide. The most common types provided to children < and > than 6 months of age are in line with the most current food allergy recommendations.⁶ Aguiar et al.¹³ evaluated 214 children assisted by Rio Grande do Norte’s Program for Evaluating, Indicating, and Using Special Infant Formulas for Children with Cow’s Milk Protein Allergy, finding that EHF and SBF were the most common types provided to children < and > 6 months of age, respectively. It should be pointed out that the programs provide EHF with and without lactose, about which no specifications have been made in the current food allergy consensus⁶ or the Ministry of Health’s CMPA Protocol.¹⁰ In clinical practice, managers reported that when physicians choose lactose-free formula it is mainly due to gastrointestinal impairment, since lactose intolerance can occur concomitantly with CMPA. Effective selection of the appropriate formula type can lower costs, an extremely important parameter for the UHS.

CMPA is difficult to diagnose since no single parameter can conclusively determine whether the child has this condition. Accurate diagnosis must follow these steps: (I) anamnesis and physical examination, observing signs, symptoms (frequency and reproducibility), and reports from parents and caregivers; (II) a restricted maternal diet for breastfed children; (III) immunoglobulin E tests, although this is more suitable for children with type I (immunoglobulin E-mediated) and mixed reactions, and should not be the only test performed; and (IV) periodic OPT based on medical follow-up to verify oral tolerance, provided that testing is performed under medical supervision in an appropriate environment with support in case of severe reactions.⁷

Table 4

Composition and routines two programs and services that dispense infant formulas for children with cow's milk protein allergy (CMPA), Brazil, 2022

	N°	%
Professionals who directly assist children with CMPA		
Allergopediatrician	6	28.6
Social worker	8	38.1
Nurse	5	23.8
Pharmacist	1	4.8
Gastropediatrician	11	52.4
General practitioner	4	19.1
Nutritionist	18	85.7
Nutrologist	2	9.5
Pediatrician	6	28.6
Psychologist	1	4.8
Nursing technician	1	4.8
None	1	4.8
Follow-up interval		
Social worker		
Registration	5	62.5
Trimestral	1	12.5
Semestral	1	12.5
Annual	1	12.5
Nurse		
Registration	2	40
Monthly	1	20
Bimonthly	1	20
Trimestral	1	20
Pharmacist		
Monthly	1	100
General practitioner		
Trimestral	2	50
Semestral	2	50
Specialist physician (pediatric allergist or gastropediatrician)		
Bimonthly	4	25
Trimestral	10	62.5
Semestral	2	12.5
Nutritionist		
Registration	1	5.5
Monthly	3	16.7
Trimestral	10	55.6
Semestral	4	22.2
Nutrologist		
Bimonthly	2	100

^a Homogeneity test: p-value = 0.002.

^b Homogeneity test: p-value = 0.004.

IgE = immunoglobulin E dosage, OPT = oral provocation test.

Table 4 (continued)

Composition and routines two programs and services that dispense infant formulas for children with cow's milk protein allergy (CMPA), Brazil, 2022

	N°	%
Pediatrician		
Monthly	1	16.7
Trimestral	3	50
Semestral	2	33.3
Nursing technician		
Monthly	1	100
Tests offered in the municipality/state (depending on the program level) ^a		
Diagnostic endoscopy	1	4.8
IgE (total and specific)	13	61.9
Skin test	6	28.6
OPT	10	47.6
None	4	19.1
Aspects considered in the diagnosis of CMPA ^b		
Exclusion diet	1	4.8
Diagnostic endoscopy	1	4.8
Laboratory tests (total IgE, fecal occult blood, etc.)	7	33.3
Clinical history	21	100
Signs and symptoms	21	100
OPT	12	57.1
OPT performed		
No	6	28.6
Yes	15	71.4
OPT obligatory		
No	11	52.4
Yes	10	47.6
Location of OPT (n = 15)		
Clinic	5	33.3
Specialty center (secondary care)	3	20
Residence	4	26.7
Hospital	8	53.3
Basic Health Unit (primary care)	2	13.3
Not informed	1	6.7
Conditions for performing the OPT (n = 15)		
Adequate	12	80.0
Inadequate	2	13.3
Not informed	1	6.7

^a Homogeneity test: p-value = 0.002.

^b Homogeneity test: p-value = 0.004.

IgE = immunoglobulin E dosage, OPT = oral provocation test.

Table 5

Challenges, suggestions and strategies pointed out by managers of programs that provide formula for children with cow's milk protein allergy, Brazil, 2022

	N°	%
Difficulties in implementing and executing the program		
Lack of standardized protocols	4	19.1
Ineffective communication with the judicial system and parents of children with CMPA	3	14.3
Lack of patient follow-up	4	19.1
Lack of structure (storage, distribution, etc.)	8	38.1
Lack of guidance on breastfeeding in CMPA and exclusion diets	3	14.3
Lack of financial resources	13	61.9
Lack of human resources	15	71.4
Lack of tests (laboratory, OPT, endoscopy, etc.)	4	19.1
High rate of misdiagnosis	2	9.5
Sensitizing managers about the need for the program	2	9.5
Other	3	14.3
Suggestions for resolving the difficulties		
Program approval by municipal or state health councils	1	4.8
Increasing the number of tests	2	9.5
Development of indicators to evaluate the program	1	4.8
Counterpart funding from the Federal Government and States	12	57.1
Hiring of health professionals	8	38.1
Creation of reference centers	2	9.5
Creation of multidisciplinary technical commissions to communicate with the judicial system	1	4.8
Bimonthly distribution of formulas	1	4.8
Continuing education for health professionals	11	52.4
Preparation of reports to managers	3	14.3
Development and updating of standardized protocols	1	4.8
Improvement of the communication with the judicial system and parents of children with CMPA	2	9.5
Improvement of the physical structure and formula distribution logistics	6	28.6
Guidance on breastfeeding in CMPA and exclusion diets	3	14.3
Performing OPT	2	9.5
Dispensing special formulas and standard formulas separately	1	4.8
Cost reduction strategies		
Monitoring of bids	2	9.5
Increased use of soy-based formula	1	4.8
Hiring of health professionals	5	23.8
Ongoing dialogue with managers about indicators and the importance of the program	2	9.5
Continuing education for health professionals	2	9.5
Development and updating of standardized protocols	13	61.9
Establishment of partnerships with breastfeeding support networks	3	14.3
Creation of multidisciplinary teams to monitor children with CMPA	5	23.8
Provision proportional to social assessment/vulnerability	4	19.1
Inclusion of OPT	2	9.5
Organization of workflows and health networks	8	38.1
Auditing the processes	1	4.8
Reduction of the age to receive formula	2	9.5

In OPT, the child is exposed to the allergen under controlled conditions and medical supervision. Parents must be instructed about the risks and procedures to be performed, and the environment must be prepared for systemic reactions. However, 2 of the managers reported that OPTs are performed without the appropriate conditions, which can involve serious risks for the patient. There is no consensus among experts about OPT, since formulas with lactose can cause gastrointestinal intolerance that can be confused with CMPA. The clinical protocol for OPT in children with CMPA involves cow's milk or standard formulas, either without (preferably) or with low amounts of lactose to avoid lactose intolerance symptoms, which could be confused with CMPA. However, experts disagree about excluding lactose. This implies a *third challenge* to implementing these programs, since specialists are unsure about which type of protocol (low or no lactose) to use for the diagnostic test.

Continued breastfeeding is the first procedure, which is to determine if the child is reacting to breast milk⁶ and, if necessary, a maternal exclusion diet should be prescribed as a diagnostic and/or treatment tool. Expanding breastfeeding support strategies is essential, both to guide the exclusion diet and to positively reinforce breastfeeding for the mother-child dyad, resulting in lower demand for formula. Brazil is recognized worldwide for its programs to encourage breastfeeding, including a human milk bank network with 224 centers and 212 collection points nationwide.¹⁸ Such expertise must be accompanied by discussion, such as the present study, to protect infants from the unnecessary use of formulas and avoid early weaning as a result of erroneous guidance about exclusion diets and other problems.

The main challenges reported by program managers were the lack of human and financial resources. The professionals most capable of diagnosing and following up these children are allergists/allergologists, pediatricians, and gastroenterologists. According to the Federal Council of Medicine's national registry of physicians, there are 1648 allergists (of whom 134 are pediatric allergists), 37,736 pediatricians, and 4949 gastroenterologists (of whom 6 are pediatric gastroenterologists) in Brazil (CFM, 2022), the majority of whom are concentrated in the southeast region.¹⁹ However, there may be more, since this list includes only professionals who registered their specialty in a Regional Council of Medicine in order to legally advertise it.

Vieira et al. found low adherence to food allergy management guidelines among Brazilian pediatricians, which is important since most children with suspected CMPA and other food allergies will be diagnosed and treated by them, without the benefit of OPT.²⁰ Nutritionists are responsible for monitoring the nutritional status of these children and, according to the Federal Council of Nutrition, there are 187,532 active nutritionists in Brazil, of whom 89,057 (47.49%) are concentrated in the southeast region.²¹ Multidisciplinary teams can provide more conclusive diagnoses and better care for children with CMPA and their parents. Hiring specialists, as well as providing continuing education for those who directly assist these children, would facilitate the diagnostic process, excluding other health conditions that could be confused with CMPA, and provide guidance about changes common to children according to age group, as well as about clinical and laboratory tests as diagnostic support tools.

The program managers reported that a lack of federal and state funding and continuing education initiatives were the most common challenges, which shows the importance of defining competencies between agencies, hiring needs, and continuing education initiatives, which can result in better care for users and the rational use of resources.

With the emergence of the COVID-19 pandemic and social distancing practices, educational processes have undergone a number of changes, including a greater number of distance learning courses and the expansion of telemedicine and digital health initiatives. Proper implementation of these tools in the post-pandemic phase can expand and complement health professional training, allowing better care for children with CMPA. This is a further challenge to improving such programs in this country.

Other suggestions, such as increasing the number of tests, performing OPT, creating reference centers, and providing guidance about breastfeeding and exclusion diets, can contribute to more accurate diagnosis. These programs can also be strengthened by the creation of multidisciplinary technical commissions to communicate with the judicial system (and consequently reduce judicialization and include formulas not yet incorporated in the UHS), the development of indicators (time in the program, costs, etc.) and reporting objectives for managers, and program approval by municipal or state health councils.

To provide an international perspective, the government websites of the USA²², Canada²³, England²⁴ and Germany²⁵, in addition to the PubMed and SciELO databases, were searched using the keywords “milk hypersensitivity”, “cow’s milk allergy”, “program evaluation”, “infant formula”, “health service accessibility” and “allergy program”. We could find no other national programs that provided special formulas for children with CMPA.

Health Canada's Food Allergen Research Program²⁶ was created in the 1990s to improve allergen labeling regulations and support research on the prevalence of food allergies, intolerance, and celiac disease. A partnership between the Australasian Society of Clinical Immunology and Allergy and Allergy & Anaphylaxis Australia, together with patient associations and other organizations, resulted in the launch of the Australian National Allergy Strategy, which aims to “improve the health and quality of lives of Australians with allergic diseases and minimize the burden of allergic diseases on individuals, their caregivers, health services, and the community”. This is the first such initiative of medical and patient associations that has managed to attract significant federal fundin.^{27,28}

The U.S. Women, Infants, and Children Nutrition Program²⁹ provides standard foods and infant formula for vulnerable and pregnant women and children ≤ 5 years of age, although no mention is made of providing special formulas for children with CMPA.

The Finnish Allergy Program^{30,31} (2008-2018), which was developed to prevent allergies and asthma, led initiatives to improve health professional training programs, performed public information campaigns through social media, and established contact with patients and the program network. After 10 years, the program resulted in a 30% reduction in direct allergy costs, 50% fewer special diets for food allergies among preschoolers, and a 45% reduction in the incidence of occupational allergies. Compared to the results of our study, 3 points stand out. The first is the Finnish program's standardization of diagnostic tests, ie, approximately 90% of the tests were performed in standardized, certified, and audited laboratories, providing greater reliability and lower cost. Similar initiatives are crucial for better organization in the UHS, which will diagnose and monitor CMPA and dispense these special formulas. Second, this program had goals, with tools and methods for measuring specific results, allowing better monitoring and evaluation. And, finally, the involvement of patients and organized civil society,

as well as providing promotional materials for professionals and the general public, allowed more effective communication.

In Finland, most of the health system is public, with a complementary private sector, which is similar to the situation in Brazil, but for a significantly smaller population (5.5 million³⁰ vs 215 million³²). There are also other differences, such as partial reimbursement for formulas and other hypoallergenic products. As of 2006, the Finnish Government began a partial reimbursement protocol only for children whose diagnosis was confirmed through OPT in a pediatric outpatient clinic, which resulted in a 70% cost reduction³⁰. Brazilian CMPA protocols¹¹ already include this condition, but nor reimbursement for OPT.

Data collection for the present report began in June 2020, but the methods were adapted due to COVID-19 emergency measures, ie, personal interviews and on-site visits were replaced with virtual meetings. Some health departments also requested that the project undergo review by their ethics committees or research foundations. However, due to the pandemic, the evaluations were delayed, since studies related to COVID-19 were prioritized. Notwithstanding, these limitations did not reduce data collection quality or the sample size.

Conclusions

The process of incorporating 3 formulas for children with CMPA into the UHS began in 2012, however, 10 years later, it has still not been completed. Thus, due to growing litigation, some states and municipalities have decided to create programs to provide AAF, EHF and SBF. The lack of state or federal funding or a specific budget in the UHS for these formulas, in addition to the vague definition of administrative responsibilities, have led program managers to use cost reduction strategies, since they are funded by local health departments. This is the first study of this type to be conducted on a national level. Understanding the experiences of programs that already provide these formulas is essential for determining their structures and the assistance they provide.

Setting the maximum age at 2 years to receive formula, promoting multidisciplinary monitoring of these children, discussion between professionals and managers about which formulas are to be included, and developing protocols for more accurate diagnosis must all be addressed to make incorporation of

these formulas more cost-effective and beneficial to users.

Although planned for all allergies and asthma, the Finnish Allergy Program has important lessons for Brazil's context, such as: defining goals and developing assessment and monitoring tools, a careful examination of the diagnostic process (ie, standardizing diagnostic tests and certifying the places where they will be carried out), continuing education for health professionals, developing educational campaigns for the public, involving patients and associations in planning discussions, and, finally, long-term planning, since these programs have a great social impact. Such strategies could be used in Brazil to improve and advance incorporation of these formulas into the UHS.

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Risk of asthma exacerbation in adolescent users of electronic nicotine delivery systems: a systematic review and meta-analysis

Risco de exacerbação de asma em adolescentes usuários de dispositivos eletrônicos de liberação de nicotina: uma revisão sistemática e metanálise

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ABSTRACT

This study aims to investigate the association between electronic cigarette use and lung disease in adolescents. A systematic review was conducted in PubMed. We used the MeSH terms “Electronic Nicotine Delivery Systems” and “Lung Diseases” as well as synonyms in the title and abstract, with the age filter “child: birth - 18 years” to search for articles related to electronic cigarette use and lung disease in adolescents. The eligibility criteria consisted of adolescent users and exposure to e-cigarettes that resulted in lung disease. The articles were selected by independent assessment, reading first the titles and abstracts, then the full text of the selected articles. The Newcastle-Ottawa Scale was used to assess study quality, and the included studies received between 5 and 7 stars. Finally, the data were extracted for meta-analysis. Initially, 61 articles were found and 6 were considered eligible, all of which were cross-sectional and applied questionnaires. The meta-analysis found a significant association between electronic cigarette use and asthma exacerbation (adjusted OR 1.44 95% CI 1.17 - 1.76). However, no studies evaluated the association with other lung diseases, including electronic cigarette or vaping product use-associated lung injury in adolescents. The meta-analysis revealed a significant association between e-cigarette use and asthma exacerbation among adolescents with chronic asthma, as well as among their previously healthy peers.

Keywords: Asthma, adolescent, electronic nicotine delivery systems, lung diseases.

RESUMO

Este trabalho tem como objetivo investigar a associação entre o uso dos cigarros eletrônicos e doenças pulmonares em adolescentes. Foi realizada uma revisão sistemática na base de dados PubMed. Os termos Mesh incluídos na busca foram “Electronic Nicotine Delivery Systems” e “Lung Diseases” e sinônimos no título e abstract, com o filtro de idade “child: birth - 18 years”, para buscar artigos relacionados ao uso de cigarros eletrônicos e doenças pulmonares em adolescentes. Os critérios de elegibilidade consistiram em: usuários adolescentes, exposição ao cigarro eletrônico e doença pulmonar como desfecho. Os artigos foram selecionados por uma revisão pareada de maneira independente, primeiramente com a leitura dos títulos e resumos, seguida da leitura integral dos artigos selecionados, os quais foram analisados pela ferramenta New Castle-Ottawa quanto sua qualidade, e receberam entre 5 e 7 estrelas. Os dados encontrados foram extraídos para a realização da metanálise. Inicialmente foram encontrados 61 artigos, sendo seis considerados elegíveis, todos transversais e com aplicação de questionários. Na metanálise foi encontrada uma associação significativa entre o uso de cigarro eletrônico e exacerbação de asma (OR ajustado 1,44; IC 95% 1,17–1,76). Não foram encontrados estudos que avaliassem a associação do cigarro eletrônico e outras doenças pulmonares, incluindo EVALI (*E-cigarette or Vaping product use-Associated Lung Injury*), em adolescentes. Na metanálise foi encontrada uma associação significativa entre exacerbações de asma e uso de cigarros eletrônicos em adolescentes com asma crônica e nos previamente hígidos.

Descritores: Asma, adolescente, sistemas eletrônicos de liberação de nicotina, pneumopatias.

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Introduction

Electronic nicotine delivery systems (ENDS), commonly referred to as electronic cigarettes (e-cigarettes), have gained popularity since their introduction in the United States in 2007.¹ These devices consist of three components: a battery, a heating coil, and a reservoir containing the liquid to be heated. The e-liquid generally contains a solvent (propylene glycol or vegetable glycerin), varying levels of nicotine, and flavorings. This mixture is heated, converted into an aerosol, and then inhaled through the mouthpiece.²

There is a special concern about ENDS, as their dissemination and consequent popularization may be affecting the nonsmoking population.² E-cigarette use has grown substantially among adolescents and young adults,³ and one explanation is that these devices have been widely publicized on digital media.¹ Unlike conventional cigarettes, e-cigarettes do not burn. Therefore, they do not produce many of the toxic products generated by conventional cigarettes.¹ For this reason, although the effects of ENDS are not well understood, some public health scholars⁴ and e-cigarette supporters claim that they would be a safe option to conventional cigarettes as well as an effective alternative for those who are trying to stop smoking.⁵

Studies reveal, however, that e-cigarettes are not risk-free. Negative effects have been demonstrated, such as increased oxidative stress, decreased cell proliferation, and DNA damage in cell cultures exposed to aerosols from e-cigarettes.² There is also a concern about known tobacco carcinogens found within the vapor of most e-cigarettes. Heating propylene glycol also generates toxic aldehydes such as formaldehyde, acetaldehyde, and acrolein.^{2,6}

E-cigarette nicotine vapor reaches the airways in the same manner as conventional cigarette vapor, which makes both products equally addictive. In addition to dependency, the neurocognitive actions of nicotine are a cause for concern. The observed effects on adolescents and young adults are especially related to reduced reflexes, attention and reasoning deficits, and mood disorders.¹ Tom Frieden, director at the Centers for Disease Control and Prevention (CDC), says adolescence is a critical period for brain development. Nicotine exposure at a young age may cause permanent brain damage, promote addiction, and lead to sustained tobacco use.⁷

Another alarming factor related to e-cigarette use, shown in a 2017 study conducted by Soneji et al., is that these devices may contribute to cigarette smoking initiation. Likelihood was 30.4% for e-cigarette users versus 7.9% for those who had never used the device. These data refute the notion that e-cigarettes are an option for smoking cessation – on the contrary, they are a risk factor for initiation.^{1,8} Also, a longitudinal study focusing on the evaluation of an adolescent population showed that e-cigarette use by nonsmokers is associated with an increased risk of them becoming conventional cigarette smokers, even after assessing demographic, psychosocial, and risk factors.⁸

In Brazil, there are no data regarding the number of young e-cigarette users, but a United States study showed that the use of this device has increased significantly among adolescents. According to the 2014 National Youth Tobacco Survey, conducted by the CDC, there are currently 4.6 million student tobacco users, and approximately half of them (2.4 million) reported e-cigarette use.⁹ The survey also found that e-cigarette use by high school students increased approximately 800% from 2011 to 2014.⁹

Regarding the trade and import of ENDS in Brazil, both were prohibited in 2009¹⁰ by the National Health Surveillance Agency (Anvisa), given the lack of knowledge about safety and efficacy of the product. However, e-cigarettes can be easily purchased from e-commerce websites and even street stores, with prices starting at R\$49.00 for the simplest models.¹

This study seeks to evaluate the occurrence of e-cigarette or vaping product use-associated lung injury (EVALI) and other lung diseases in adolescents.

Methods

Data source and search strategy

The PubMed database was searched using specific descriptors and synonyms found in the title and abstract, based on the following Medical Subject Headings (MeSH) terms: “electronic nicotine delivery systems,” “lung diseases,” “child,” “pediatrics,” “birth – 18 years,” and variations. The search strategy was limited to the pre-pandemic period, as the new coronavirus pandemic could influence the findings and deserves to be evaluated separately. Only articles written in English were considered. No search filters were used for the date of articles published until 2020.

Study selection

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (Prisma) checklist recommendations.¹¹ The process of article selection began with removal of duplicates; then, two raters independently screened the titles and abstracts; subsequently, the pair of raters came to an agreement for choosing the articles that fit the eligibility criteria. When there was no consensus, a third rater was consulted for making the decision. The previously selected articles were then screened in full text and, finally, were elected for the review. The eligibility criteria were adolescent users, exposure to e-cigarettes, and, as an outcome, lung disease.

Inclusion and exclusion process

Randomized and nonrandomized cross-sectional studies reporting analyses of respiratory conditions in adolescent ENDS users were included. Incomplete texts and studies related to diagnosis of diseases other than respiratory diseases were excluded. Articles that evaluated consequences other than the respiratory outcome and those that addressed exposure to tobacco products other than ENDS were also excluded.

Data extraction

As recommended by the Prisma checklist,¹¹ two raters extracted and analyzed data from the selected articles. Authorship, year of publication, nationality, study design, sample size, sex, age group, and other epidemiological data were extracted from the selected studies.

Quality assessment

Two independent raters assessed the methodology of the selected studies using the New Castle-Ottawa Scale (NOS),¹² which was designed to score the quality of nonrandomized studies between 0 and 9 stars. NOS can be used either as a checklist or a scale. A Delphi process was used to develop the tool, which was then tested in systematic reviews and refined. It consists of eight items, divided into three categories, including selection, comparability, and, depending on study type, outcome (cohort studies) or exposure (case-control studies). For each item, a series of response options is provided. A star system is used to allow a semiquantitative assessment of

study quality, so that studies receive one star for each item if they meet the requirement for that item, except for the comparability item, which allows two stars being assigned.¹²

Results

In total, 257 articles were found during initial database search with the descriptors “electronic nicotine delivery systems” and “lung diseases,” all in English language. After the filter for the desired age group was applied, 74 articles were obtained. Thirteen duplicates were excluded, and then the screening process for the 61 selected articles began (Table 1).

Full-text articles were screened using the Prisma method,¹¹ as previously reported, and 55 articles were excluded from the final analysis for one of the following reasons: including other age groups; evaluating e-cigarette consequences other than the respiratory outcome; including tobacco products other than e-cigarettes; and, in some cases, not being available in full text. Therefore, 6 articles were selected in the eligibility analysis and were then submitted to the New Castle-Ottawa Scale,¹² which scores the quality of nonrandomized studies between 0 and 9 stars (Figure 1).

The studies selected for this systematic review received between 5 and 7 stars, were all cross-sectional, and administered questionnaires as part of their methodology, as shown in Table 2.

A single article – Bayly (2019)¹³ – received 5 stars and, for this reason, was not included in the meta-analysis. Schweitzer (2017)¹⁴ received 6 stars, and the others – Cho (2016),¹⁵ Choi (2016),¹⁶ Kim (2017),¹⁷ and McConnell (2017)¹⁸ – received 7 stars.

The data reported in the 5 articles included in the final sample were extracted into a table, for subsequent systematic review and meta-analysis. The meta-analysis consisted of an analysis of data expressed as odds ratio (OR).

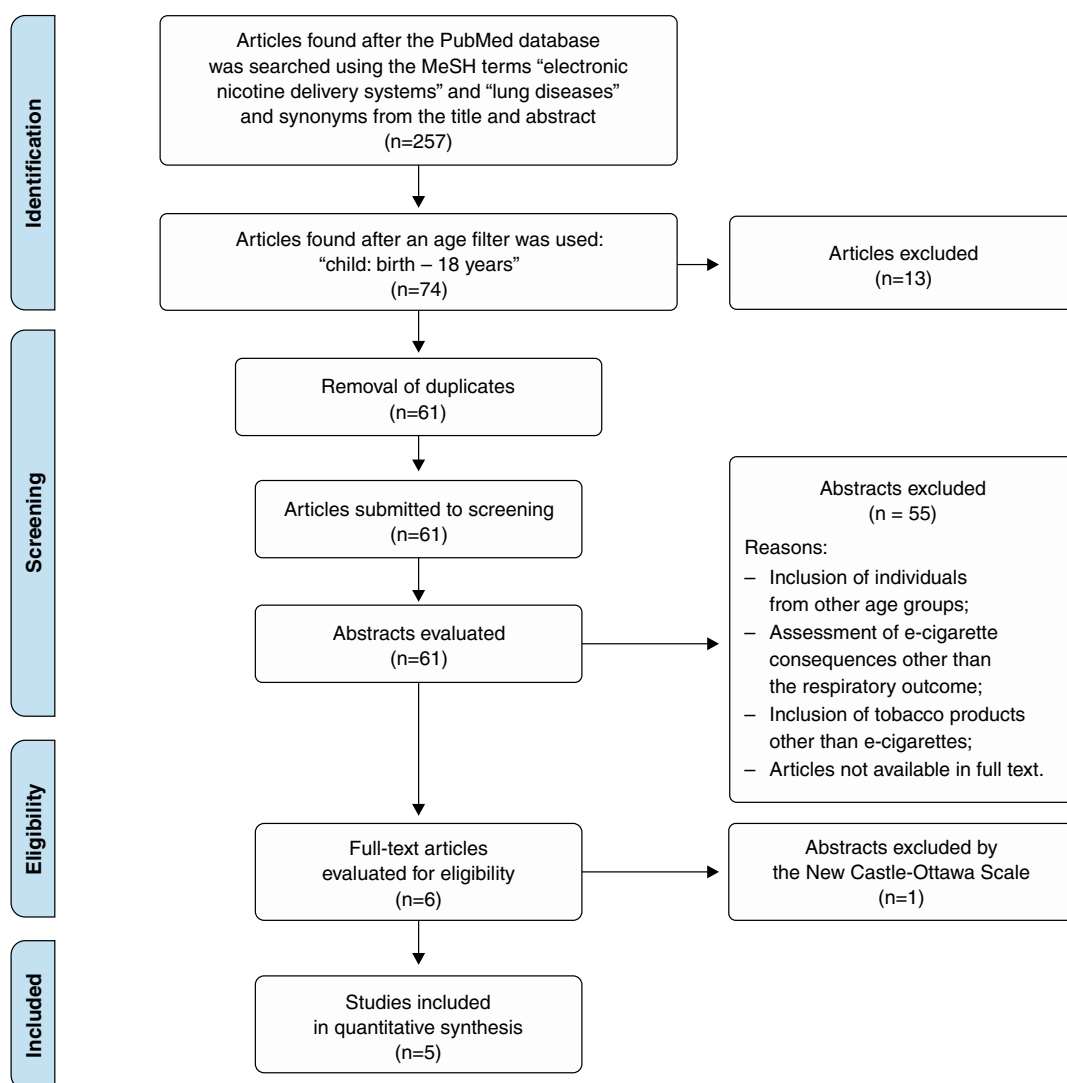
Most studies reported a population of adolescents aged 11 to 18 years, with the same proportion of female and male participants. Two studies were conducted in South Korea, and the rest in the United States, in the states of Florida and California. Four studies evaluated adolescents with a previous diagnosis of asthma, while the others assessed healthy people. Only 2 studies asked the adolescents about previous tobacco use. Three articles discussed sociodemographic issues, such as ethnicity, physical activity, parents'

Table 1

Description of inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Studies evaluating age group up to 18 years	Language other than English
Studies evaluating vaping use	Age groups other than children and adolescents
Articles evaluating respiratory outcomes	Articles evaluating outcomes other than respiratory outcomes
Detailed description of methods used	Tobacco products other than ENDS
	Studies not available in full text or available in other forms (abstract, thesis, dissertation)

ENDS = electronic nicotine delivery systems.

**Figure 1**

Prisma algorithm for study selection process

educational level, socioeconomic status, and region of residence. The 6 studies reported, as an outcome, asthma exacerbation in people previously diagnosed with asthma and asthma symptoms in previously healthy people.

The meta-analysis found a significant association between e-cigarette use and asthma exacerbation (adjusted OR 1.44; 95% CI 1.17-1.76). Five studies were statistically relevant, and all showed that e-cigarette use may exacerbate asthma. Only one study – McConnell et al. – crossed the vertical line in the forest plot, which reduced its statistical relevance for the research (Figure 2).

Discussion

The number of e-cigarette users has grown alarmingly around the world, particularly among young people.¹ In the United States, these devices have been addressed as a serious public health problem in recent years.^{1,3} Although epidemiological data referring to Brazil are lacking and free dissemination and marketing of these products are banned, the perception that vaping use has been increasing among Brazilian youth is undeniable.^{10,19,20}

One suggested explanation for such growth is the mistaken notion that these devices are harmless. Also, e-cigarettes have become synonymous with social acceptance and ostentation among adolescents and young adults.^{9,20}

Although the effects of ENDS are not well understood, it is known that they are an important risk factor for cardiovascular and lung diseases.²¹ In addition to chemical dependency, there are some concerns about the deleterious neurocognitive actions of nicotine, confirmed production of carcinogens, and effects on the vascular endothelium with a consequent impact on cardiovascular diseases.^{2,6,7,21}

Compared to conventional cigarettes, e-cigarette vapor reaches the airways in the same manner and is equally addictive.^{2,6,7}

Regarding local aggressive action on the respiratory epithelium, many studies indicate specific inflammatory effects and confirm the impairment of innate immune response in the lungs. Exposure to ENDS flavorings results in abnormal activation of lung epithelial cells and β -defensins, dysfunction of macrophage phagocytic activity, increased mucin levels (MUC5AC), and abnormal activation of neutrophil response (NETosis).^{22,23} Menthol flavorings disrupt

Table 2
Scores of selected studies in the New Castle-Ottawa Scale

	Selection			Comparability		Outcome		
	Representa- tiveness of the sample	Sample size	Non- respon- dents	Ascertain- ment of the exposure	Compara- bility	Assessment of the outcome	Statistical test	Total (9/9)
McConnell et al., 2017 ¹⁸	★	★	0	★	★★	★	★	7/9
Choi K et al., 2016 ¹⁶	★	★	0	★	★★	★	★	7/9
Cho JH et al., 2016 ¹⁵	★	★	0	★	★★	★	★	7/9
Bayly JE et al., 2019 ¹³	★	★	0	★	0	★	★	5/9
Kim SY et al., 2017 ¹⁷	★	★	0	★	★★	★	★	7/9
Schweitzer RJ et al., 2017 ¹⁴	★	★	0	★	★	★	★	6/9

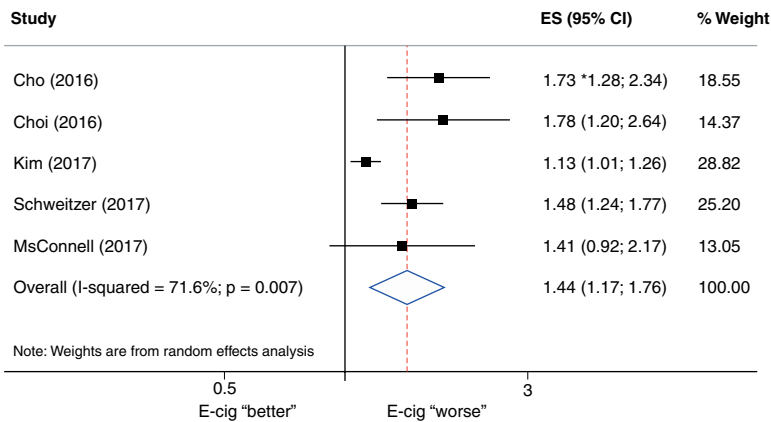


Figure 2
Meta-analysis of asthma exacerbation and e-cigarette (e-cig) use

innate immune responses and may be associated with allergies and asthma through activation of the transient receptor potential ankyrin 1 (TRAP1).²³ A potential effect on the respiratory microbiota of ENDS users has also been studied.²³

More recently, studies showing a positive association between ENDS use and viral respiratory infections have gained prominence. Cigarette users showed a consistent change in antiviral response, with a decreased production of defense cytokines (IFN γ , IL-6, and IL-12p40) when exposed to live-attenuated influenza virus, and failed to increase influenza-specific IgA levels, significantly differing from the nonsmoking group.²⁴

This study reported a relevant finding: an increased risk of asthma exacerbation in e-cigarette users, which was detected in those with chronic asthma but also in healthy adolescents.

Asthma is one of the most common chronic diseases of childhood and adolescence, with increasing prevalence, high number of admissions, and high social burden.²⁵ It is a worldwide health problem that affects approximately 300 million individuals. Twenty million live in Brazil, accounting for a prevalence of 10% of the population. The rate increases when it comes to school-aged children and adolescents, reaching up to 20%.²⁵ The negative impact of exposure to conventional cigarette smoke on asthma symptoms has already been proven.

Asthmatic children and adolescents exposed to passive smoking have moderately severe asthma, require inhaled corticosteroids more often, and show a greater frequency of daytime symptoms compared to those who are free from exposure.²⁶

It is not difficult to imagine that, because asthma is a disease that causes inflammation of the airways, environmental exposure to allergens and irritating agents such as cigarette smoke generates hyper-responsiveness, with reduced ventilatory flow and consequent exacerbation of the disease.^{25,26} The present study showed that heated vapor and e-cigarette-generated products have the same ability to trigger a dreaded asthma exacerbation.

A study evaluating immediate local effect showed an increase in airway resistance at 5 Hz and at 20 Hz, measured by impulse oscillometry, after use of an e-cigarette for 5 minutes. This effect was also described for use of conventional cigarettes, suggesting that they are potential constrictors.²⁷ The study found a significant increase in oxidative stress in the e-cigarette group, proven by a decrease in fraction of exhaled nitric oxide.²⁷

EVALIs were not found in this systematic review when the age group was restricted to adolescents. However, recent studies showing a positive association between ENDS use and a positive COVID-19 diagnosis revealed that young users of e-cigarettes only were 5 times more likely (95% CI: 1.82-13.96) of

having a positive diagnosis; when both e-cigarettes and conventional cigarettes were used, likelihood was even greater, almost 7 times (95% CI: 1.98-24.55), compared to young nonsmokers.²⁸

The results of this review and meta-analysis indicate that e-cigarette use causes a real harm in adolescents. Preventive measures that ensure education, information, and action plans to fight e-cigarette use must be adopted by educators, health professionals, and managers, aiming to stop the growing adherence to these devices by young people.

Pediatricians and other professionals who assist adolescents in daily practice need to provide the asthmatic population with special attention, which includes investigating e-cigarette use during history taking and giving firm and precise guidance on the harm that these devices may cause.

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Comparative effects of aerobic, combined, and intermittent exercise in children and adolescents with asthma: a systematic review

Efeitos comparativos dos exercícios aeróbicos, combinados e intermitentes em crianças e adolescentes com asma: uma revisão sistemática

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ABSTRACT

Asthma is a chronic disease with a heterogeneous pathogenesis and etiology. Inflammation is considered one of the main triggers of bronchospasm and airway obstruction, which lead to clinical manifestations. Exercise is encouraged as a way to avoid cardiovascular, musculoskeletal, and psychosocial disorders and help control the disease. Our objective was to analyze the comparative effects of aerobic, combined (resistance and aerobic), and intermittent exercise on the functional capacity, lung function, and quality of life of children and adolescents with asthma, as well as to identify the relationship between exercise, other functional parameters, and inflammatory components characteristic of asthma. This was a systematic review of randomized clinical trials involving asthmatic populations aged between 6 and 17 years. PEDro, Lilacs, PubMed, and Cochrane Library/Central were searched for studies published in the last 10 years in English or Portuguese. We identified 8136 references, of which only 3 met the eligibility criteria. Considering the intervention time, aerobic exercise showed better functional capacity results. Regarding the common parameters used to assess lung function, intermittent exercise was the only type to result in significant change. Regarding quality of life, intermittent exercise had superior results, while combined exercise stood out for musculoskeletal improvement. Exercise can be a good non-pharmacological strategy for reducing the consequences of this pathology.

Keywords: Asthma, exercise, child, adolescent.

RESUMO

A asma é uma doença crônica, com patogênese e etiologia heterogêneas. Considera-se o quadro inflamatório como um dos principais desencadeantes do broncoespasmo e obstrução das vias aéreas, os quais levam às manifestações clínicas. Sugere-se que os exercícios físicos sejam uma terapêutica importante para evitar distúrbios cardiovasculares, musculoesqueléticos e psicossociais, e ajudar no controle da doença. O objetivo deste estudo é analisar os efeitos comparativos dos exercícios físicos aeróbicos, combinados (resistência e aeróbico) e intermitentes na capacidade funcional, função pulmonar e qualidade de vida das crianças e adolescentes com asma, identificando também as possíveis relações dos exercícios com outros parâmetros funcionais e componentes inflamatórios característicos da asma. As fontes de buscas utilizadas foram PEDro, LILACS, PubMed e Cochrane Library/Central, dos quais selecionou-se apenas ensaios clínicos randomizados, em crianças e adolescentes com o diagnóstico de asma, com idade entre 6 e 17 anos, publicados nos últimos dez anos, nas línguas inglesa e portuguesa. Foram identificadas 8.136 referências, sendo que destas, apenas três preencheram os critérios de inclusão. Considerando o tempo de intervenção, o exercício aeróbico apresentou melhores resultados referente à capacidade funcional. Em relação ao parâmetro comum utilizado para avaliar a função pulmonar, o exercício intermitente foi o único a obter alterações significativas. Quanto à qualidade de vida, o exercício intermitente obteve resultados superiores, e os exercícios combinados destacam-se pela melhora musculoesquelética. A prática de exercícios físicos pode ser uma boa estratégia, não farmacológica, para reduzir as consequências desta patologia.

Descritores: Asma, exercício físico, criança, adolescente.

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Introduction

Asthma, a chronic non-communicable disease, is common in children and adults. Its main manifestation is respiratory symptoms, such as wheezing, coughing, dyspnea, and chest tightness. These result from an inflammatory condition that leads to bronchospasm and airway obstruction.¹⁻⁴ Nevertheless, asthma has a heterogeneous pathogenesis and etiology that can result in different clinical presentations.^{1,2,5}

The allergic (atopic) form is commonly diagnosed in childhood and may be associated with individual susceptibility or genetic or environmental factors. On the other hand, non-allergic (non-atopic) asthma is often associated with later diagnosis, generally after 40 years of age, and may be persistent or intermittent. Viral infections of the respiratory system, low temperatures, and physical activity can initiate both forms of crisis, but exposure to allergens is only associated with the allergic form.⁶⁻¹⁰

In the allergic form, dendritic cells can initiate an immune response, processing and presenting an antigen to type 2 helper T cells, which induces production of interleukins (IL-4, IL-5, IL-13).^{2,5,10-12} These cytokines regulate other receptors and stimulate the production of eosinophils, mast cells, and basophils. The inflammatory condition can be mediated by histamines, prostaglandins, and leukotrienes, which trigger greater smooth muscle contractility, vascular congestion, and infiltration, obstructing the airflow.⁴ In the non-allergic form, innate lymphoid cells stimulate the production of interleukins, and the process results from epithelial damage by pollutants or microorganisms, such as viruses.^{5,9}

The relationship between a persistent inflammatory condition, oxidative stress, and reactive oxygen species is an important pathophysiological component, whose role in the activation of inflammatory mediators of the immune system produces greater reactivity of the respiratory system, lipid peroxidation, and increased vascular permeability.^{10,15-18}

Among children, asthma is more prevalent in boys, but this changes in adolescence, with an increasing prevalence in girls.^{7,19} Disease severity can be classified as persistent (41%), periodic (38%), or in remission (21%).⁵ In 2015 the asthma prevalence in Brazil among 5- to 19-year-olds was 16%, while in 2020 there were an estimated 15,406 hospitalizations due to crises, costing a total of BRL 9,385,208.68.²⁰ Thus, asthma must be considered a public health problem, since it is responsible for many

hospitalizations each year, whose expense impacts the health care system.^{7,19,21,22}

Diagnosis should be based on clinical findings. Symptoms and their inflammatory characteristics should be correlated with their frequency, as well as symptomatic response to specific medications. Lung function testing contributes to diagnosis, verifying limitations in expiratory airflow. These limitations are associated with a lower than expected ratio between forced expiratory volume in one second (FEV₁) and forced vital capacity according to sex, age, race, and height.²²⁻²⁴

For preschool-aged children, diagnostic confirmation can involve greater challenges due to heterogeneous inflammatory conditions associated with other pathologies and difficulty performing the lung function test. In this age group, it is still common to find an absence of symptoms for 1 year (remission), 5 years, or normal spirometry values.²⁵⁻²⁷

Exercise-induced bronchoconstriction due to bronchial hyperresponsiveness may be the only symptom in some individuals.²⁸⁻³⁰ An increased sensation of dyspnea during exercise may be one reason why asthma patients avoid it, which could also be associated with the patient's condition at rest. However, a sedentary lifestyle can trigger a vicious cycle in which cardiovascular and musculoskeletal disorders are associated with lung impairment. Thus, prolonged inactivity can aggravate the patient's baseline condition.³¹⁻³³

In the pediatric population, asthma can lead to functional and social impairment, which, when persistent, affect their adult lives. Exercise has been suggested as an important non-pharmacological therapy for controlling the disease, improving functional capacity, lung function, and quality of life. Correct prescription of exercise intensity, frequency, and duration can directly influence the results.³⁴⁻³⁸ One study demonstrated that an exercise program reduces bronchial hyperresponsiveness and the concentration of some pro-inflammatory biomarkers, contributing to lower exacerbation and improved quality of life through better clinical control.³⁹ The changes triggered by this pathology and its prevalence make the topic relevant.

The present study aimed to analyze the comparative effects of aerobic, combined (resistance and aerobic), and intermittent exercise on functional capacity, lung function, and quality of life in children and adolescents, in addition to identifying relationships between exercise

and other functional parameters and inflammatory components characteristic of asthma.

Methods

Protocol and registration

This systematic review was registered in the international prospective register of systematic reviews (number CRD42021252567) and was conducted according to Preferred Reporting Items for Systematic reviews and Meta-Analyses⁴⁰ and Cochrane Handbook for Systematic Reviews of Interventions⁴¹ guidelines.

Eligibility criteria

The inclusion criteria were randomized clinical trials of children and adolescents (aged 6 to 17 years) diagnosed with asthma that were published in English or Portuguese the last 10 years. The exclusion criteria were protocols for randomized clinical trials, trials without a control group or a control group who did not receive usual care, trials with adults, exclusive comparisons between medication types, comparisons between medication and exercise-induced bronchoconstriction, and duplicate publications.

Searches were performed in the Physiotherapy Evidence Database (PEDro), Lilacs (Bireme/BVS), PubMed (MEDLINE), and Cochrane Library/Central, with the final search occurring on May 15, 2021. To guide the searches, a PICO question was developed: population (children and adolescents diagnosed with asthma), intervention (aerobic, combined, and intermittent physical exercise), comparisons (control group), and outcomes (functional capacity, lung function, quality of life). The search strategy for PubMed (MEDLINE) was as follows:

Population: "Asthma" [Mesh] OR "Asthmas" OR "Bronchial Asthma" OR "Asthma, Bronchial".

Intervention: "Exercise" [Mesh] OR "Exercises" OR "Physical Activity" OR "Activities, Physical" OR "Activity, Physical" OR "Physical Activities" OR "Exercise, Physical" OR "Exercises, Physical" OR "Physical Exercise" OR "Physical Exercises" OR "Acute Exercise" OR "Acute Exercises" OR "Exercise, Acute" OR "Exercises, Acute" OR "Exercise, Isometric" OR "Exercises, Isometric" OR "Isometric Exercises" OR "Isometric Exercise" OR "Exercise, Aerobic" OR "Aerobic Exercise" OR "Aerobic Exercises" OR "Exercises, Aerobic" OR "Exercise Trainings" OR

"Training, Exercise" OR "Trainings, Exercise" OR "Circuit-Based Exercise" [Mesh] OR "Circuit Based Exercise" OR "Circuit-Based Exercises" OR "Exercise, Circuit-Based" OR "Exercises, Circuit-Based" OR "Circuit Training" OR "Training, Circuit" OR "Resistance Training"[Mesh] OR "Training, Resistance" OR "Strength Training" OR "Training, Strength" OR "Weight-Lifting Strengthening Program" OR "Strengthening Program, Weight-Lifting" OR "Strengthening Programs, Weight-Lifting" OR "Weight Lifting Strengthening Program" OR "Weight-Lifting Strengthening Programs" OR "Weight-Lifting Exercise Program" OR "Exercise Program, Weight-Lifting" OR "Exercise Programs, Weight-Lifting" OR "Weight Lifting Exercise Program" OR "Weight-Lifting Exercise Programs" OR "Weight-Bearing Strengthening Program" OR "Weight-Bearing Strengthening Program" OR "Strengthening Program, Weight-Bearing" OR "Strengthening Programs, Weight-Bearing" OR "Weight Bearing Strengthening Program" OR "Weight-Bearing Strengthening Programs" OR "Weight-Bearing Exercise Program" OR "Exercise Program, Weight-Bearing" OR "Exercise Programs, Weight-Bearing" OR "Weight Bearing Exercise Program" OR "Weight-Bearing Exercise Programs" OR "High-Intensity Interval Training" [Mesh] OR "High Intensity Interval Training" OR "High-Intensity Interval Trainings" OR "Interval Training, High-Intensity" OR "Interval Training, High-Intensity" OR "Training, High-Intensity Interval" OR "Trainings, High-Intensity Interval" OR "High-Intensity Intermittent Exercise" OR "Exercise, High-Intensity Intermittent" OR "Exercises, High-Intensity Intermittent" OR "High-Intensity Intermittent Exercises" OR "Sprint Interval Training" OR "Sprint Interval Trainings"

Comparisons: randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [pt] OR evaluation studies as topic [mh] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])

Outcomes: outcomes were not included in the searches to expand the findings.

In the other databases, terms corresponding to the MeSH terms were used: "Asthma", "Aerobic Exercise",

“Circuit Training”, “Resistance Training” and “High-Intensity Intermittent Exercise”.

Data analysis and extraction

The articles were initially analyzed in EndNote to exclude duplicate references. Language, year, and study type filters were then applied. The titles and abstracts of the remaining articles were read, and those meeting the eligibility criteria were analyzed in full. The process was performed by 2 independent and blinded reviewers. Disagreements were resolved by consensus, or when necessary, a third reviewer. The data were extracted using a form filled out by independent and blinded reviewers. Data synthesis was performed qualitatively, using a comparative table for the results.

Risk of bias

Methodological quality was assessed according to the Cochrane Handbook for Systematic Reviews of Interventions⁴¹: random sequence generation, allocation concealment, participant and personnel blinding, outcome evaluation blinding, incomplete outcome data, selective reporting, and others sources of bias. Studies were classified as high, unclear, or low risk of bias. Study quality was assessed independently by 2 blinded reviewers.

Results

Study selection

The electronic database search identified 8,136 records, of which only 3 met the inclusion criteria. The search method is shown in the inclusion flowchart (Figure 1), as are the methodological criteria used to exclude certain studies.

Study characteristics

The aggregate sample of the included articles was 185 participants (93 interventions and 92 controls). The mean age in the control and intervention groups was 11 years. The sample sizes ranged from 27 to 105 individuals. In all 3 studies, the control group received usual care. Two of the studies scored 642.43 and the other scored 544 on the PEDro scale (Table 1).

Two studies^{42,44} used Global Initiative for Asthma criteria to classify asthma, while the other did not specify which criteria were used.⁴³ Two studies classified disease severity from mild to severe^{42,43}

and the other as controlled to uncontrolled.⁴⁴ The participants showed moderate and severe,⁴² controlled and partially controlled,⁴⁴ and mild and moderate asthma.⁴³ The baseline FEV₁ obtained through spirometry in 1 study⁴² was 1.81 (SD, 0.58) in the control group and 1.96 (SD, 0.62) in the intervention group ($p = 0.546$). In another study,⁴³ the control group value was 1.91 (0.95), while the intervention group value was 2.44 (0.55) ($p = 0.072$). FEV₁ in the third study⁴⁴ was 1.53 (0.79) in the control group and 1.33 (0.54) in the intervention group ($p = \text{not significant}$). Lack of exacerbation or change in medication was a criterion in all 3 studies, and 2 (66.7%) specified which medications were used (Table 2).

The applied techniques included aerobic training ($n = 1$), intermittent training ($n = 1$), and a combined exercise program ($n = 1$). All of the studies evaluated functional capacity, lung function, and quality of life outcomes in children and adolescents with asthma. In addition to these outcomes, 1 study assessed inflammatory component and respiratory muscle strength,⁴² 1 study assessed body composition,⁴⁴ and 2 studies assessed the control and perception of asthma^{42,43} (Table 3).

Synthesis of the results

In the study involving aerobic training,⁴² no significant differences were found in spirometric variables, such as FEV₁ and forced vital capacity. Increased peak expiratory flow (PEF), improved functional capacity, and improved quality of life were found in the exercise group. Since there were no changes in plasma cytokines produced by Th1, Th2 and Th17, there was no improvement in inflammatory component. The sensation of dyspnea at the end of the protocol was significantly better in the intervention group (0.7 [SD, 0.3]; $p < 0.01$) than the control group (3.2 [SD, 0.3]; $p < 0.01$).

Significant changes were found in all analyzed parameters in the intermittent training intervention protocol.⁴⁴ Unlike the study that used aerobic intervention,⁴² the one involving intermittent exercise⁴⁴ found increased FEV₁. BMI in the intervention group reduced from 19.69 kg/m² ($p = 0.049$) to 17.49 kg/m² ($p < 0.001$) after the program, while that of the control group did not improve. Another significant result that demonstrates the influence of exercise on the cardiorespiratory system was heart rate, which reduced from 141.26 bpm to 115.16 bpm ($p < 0.001$)

after the program in the intervention group. Perceived exertion (Borg scale) reduced from 5.15 to 0.73 ($p < 0.001$) after the program in the intervention group.

Using combined exercise, one study found improved lung function, ie, decreased ventilatory

equivalent for oxygen (VE/VO_2 ratio) and an increase in peak oxygen consumption (VO_2 peak)⁴³. Regarding resistance exercise, muscle strength improved from 80% to 92% in the intervention group and from 41% to 62% in the control group. The results are shown in Table 4.

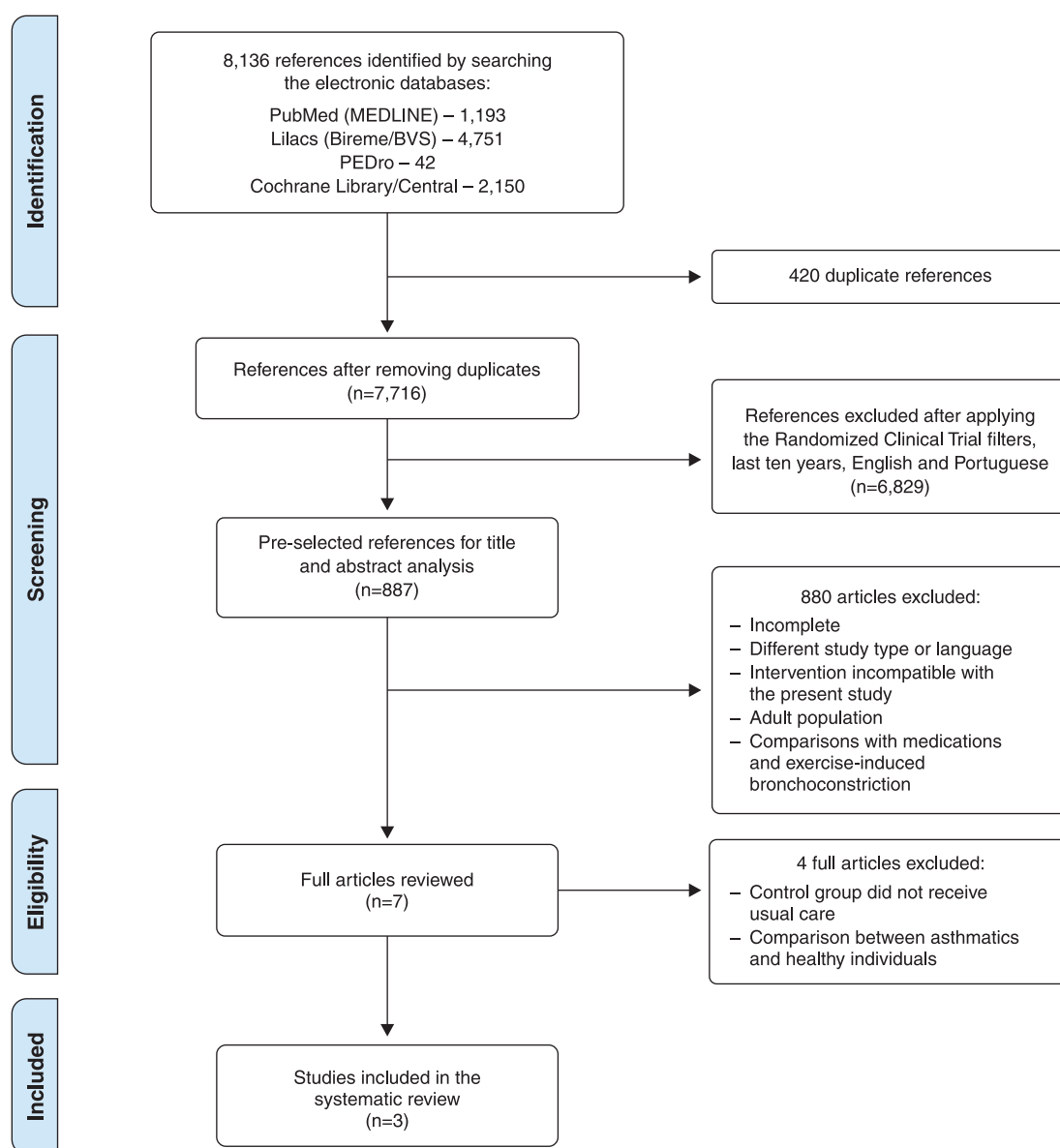


Figure 1
Flowchart of studies

Table 1

Characteristics of the included studies

Author/year	Number of participants	Mean age	Control group Characteristics	Clinical Trial Registration	PEDro score
Andrade et al., 2014	CG (n=17) IG (n=10)	CG (11.4 ± 2.3) IG (11.7 ± 2.3)	Usual routine	NCT0192052	6/10
Latorre-Román et al., 2014	CG (n=47) IG (n=58)	CG (11.51 ± 1.42) IG (11.55 ± 1.01)	Usual care	X	5/10
Sanz-Santiago et al., 2020	CG (n=28) IG (n=25)	CG (11.1 ± 2.9) IG (12.1 ± 2.1)	Clinical guideline routine	NCT02693301	6/10

CG = Control Group, IG = Intervention Group.

Table 2

Classification of disease and medications used in the included studies

Study	Classification	Severity	Pulmonary function	Medications
Andrade et al., 2014	GINA	Moderate GC: 16 GI: 9 Severe GC: 1 GI: 1	FEV ₁ (l) CG: 1.81 (0.58) IG: 1.96 (0.62)	Inhaled corticosteroids Long-acting beta-2 agonist
Latorre-Román et al., 2014	GINA	Controlled GC: 57,4% GI: 58,6% Partial controlled GC: 42,6% GI: 41,4,6%	FEV ₁ (l) CG: 1.53 (0.79) IG: 1.33 (0.54)	X
Sanz-Santiago et al., 2020	X	Mild to moderate	FEV ₁ (l) CG: 1.91 (0.95) IG: 2.44 (0.55)	Inhaled corticosteroids Long-acting beta-2 agonist Leukotriene receptor antagonists Oral corticosteroids Omalizumab

CG = Control Group, IG = Intervention Group, FEV₁(l) = Forced expiratory volume in one second (liters).

Risk of bias

The risk of bias in each study (Table 5) and the risk of bias across studies (Figure 2) were determined according to the Cochrane Handbook for Systematic Reviews of Interventions.⁴¹

Discussion

The consensus of the included studies was that asthma symptoms and fear of shortness of breath in asthmatic children, in addition to related psychosocial aspects, can lead to low exercise tolerance and result in sedentarism, which directly influences cardiorespiratory fitness and peripheral muscle.

Hence, exercise has the potential to improve symptoms and exacerbation, lung and cardiovascular function, and quality of life. However, 2 studies^{42,43} reported difficulties correlating the influence of exercise on inflammatory parameters, peripheral muscle strength, and lung function, and determining whether the results occurred in isolation or were associated, in a cause-consequence relationship.

In 1 study,⁴⁴ the authors stated that exercises which help cardiopulmonary function and lower limb muscle strength should be prioritized in training protocols. High-intensity exercise resulted in greater gains than low-intensity exercises, but a combination of these (intermittent) was recommended because

Table 3

Intervention protocols and main outcomes

Study	Intervention	Duration	Frequency	Intensity	Session time	Outcomes
Andrade et al., 2014	Supervised aerobic training on an electric treadmill	6 weeks	3 times a week	Warm up and cool down (40%-50% of maximum heart rate) Exercise (70%-80%)	10 min of warm up, 20 min of training (first and second week), 30 min of training (third to sixth week), 5 min of cool down	Inflammatory component, functional capacity, respiratory muscle strength, quality of life, and symptom perception
Latorre-Román et al., 2014	Supervised intermittent exercise (alternating low and high intensity)	12 weeks	3 times a week	Borg scale 0 to 10 (low intensity to high intensity)	10 min of warm up, 40 min of exercise alternating strength, aerobic and anaerobic, 10 min of cool down	Lung function, functional capacity, body composition, and quality of life
Sanz-Santiago et al., 2020	Supervised combined exercise (resistance and aerobic)	12 weeks	3 times a week	The workload for each session corresponded to HR at the first ventilatory threshold. For resistance exercises, initially 40% of 5-RM was performed, progressing to 60%	10 min of warm up on a cycle ergometer, 20 to 40 min training on a cycle ergometer, resistance exercise (approximately 20 s each set)	Cardiorespiratory capacity, muscle strength, lung function, of aerobic quality of life, asthma control, and functional capacity

Table 4

Comparisons of lung function, functional capacity and quality of life variables

Study	Lung function	Functional capacity	Quality of life
Andrade et al., 2014	PEF reduced by 20.6 L/min in the CG and increased by 34.5 L/min in the IG Increased respiratory muscle strength only in the IG (22.6 cmH ₂ O MIP and 21 cmH ₂ O MEP) No significant differences in FEV ₁ or FVC between groups	6MWT: 99.7 m increase in the IG; no change in the CG	PAQLQ: IG increased 1.2 (p<0.001); CG decreased 0.1 (p=0.642)
Latorre-Román et al., 2014	PEF: mean pre-intervention value 0.84 L/s increased to 1.56 L/s post-intervention in IG FEV ₁ increased by 1.22 L in the IG	6MWT: 176.49 m increase in the IG	PAQLQ: IG increased 2.51 (p<0.001)
Sanz-Santiago et al., 2020	Increased VO ₂ peak and reduced VE/VO ₂ No significant differences in FEV ₁ or FVC between groups	Treadmill test: IG duration increased compared to the CG No significant differences were found in the functional tests (agility walking and in stairs)	No significant differences were found in either group

CG = Control Group, IG = Intervention Group, FEV₁ = Forced expiratory volume in one second, PEF = peak expiratory flow, VE/VO₂ = Ventilatory Equivalents for Oxygen, VO₂ peak = peak oxygen consumption, HR = heart rate, 6MWT = 6 Minute Walk Test, FVC = Forced vital capacity, PAQLQ = Pediatric Asthma Quality of Life Questionnaire, MIP = Maximal inspiratory pressure, MEP = Maximal expiratory pressure.

it provides a tolerable training load for beginners. Another study⁴³ suggested that associating aerobic and resistance exercises would be the best treatment strategy for improving cardiorespiratory and muscle function. However, adequate volume and different exercise modalities are necessary. One study⁴² found that aerobic exercise alone led to improved functional capacity, but presented variable results in terms of quality of life and inflammatory component.

Regarding intervention time, the aerobic training protocol⁴² showed better PEF results, with a mean difference of 0.575 liters/second, compared to the intermittent training study,⁴⁴ whose mean difference

was 0.72 liters/second. The aerobic exercise group⁴² also had better results in the 6-minute walk test than the intermittent intervention group.⁴⁴ Considering quality of life, intermittent training⁴⁴ led to better results than aerobic⁴² or combined exercise.⁴³ The intermittent training protocol⁴⁴ was the only one that led to a significant change in FEV₁, an outcome common to all studies. Other parameters could not be compared, due to measurement differences or lack of certain outcomes.

One limitation of this review was the fact that only 3 studies were included, resulting in a small sample size. We also point out the scarcity of studies

Table 5

Risk of bias in each included study

Study	Random sequence generation	Allocation sequence concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Andrade et al., 2014	Low	Low	Low	High	Low	Low	Low
Latorre-Román et al., 2014	Low	Unclear	Unclear	High	Low	Low	Low
Sanz-Santiago et al., 2020	Low	Low	Unclear	High	Low	Low	Low

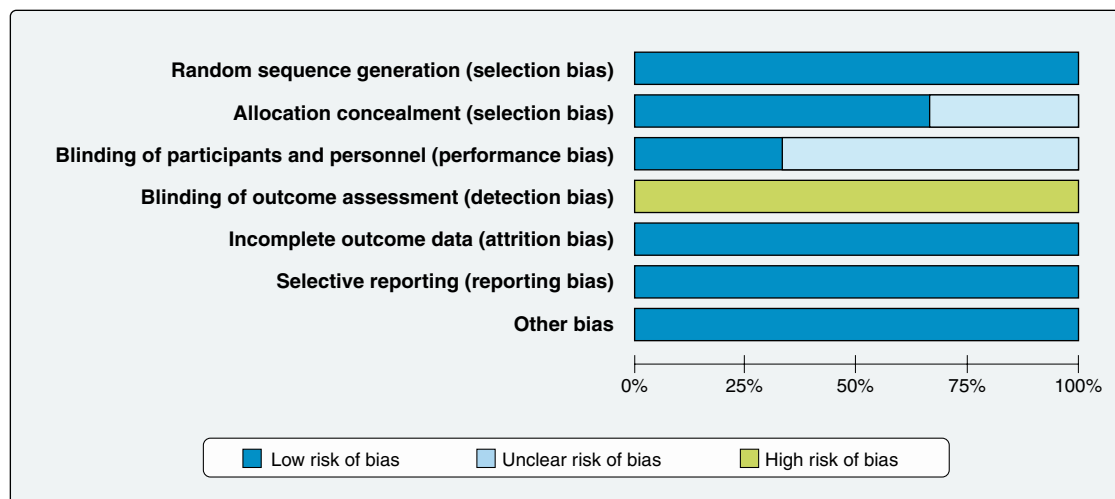
Low = study has the least bias, and results are considered valid.

Unclear = study may be missing information, making it difficult to assess limitations and potential problems.

High = rating indicates significant bias that may invalidate the results (at least one-half of the individual quality items are rated high risk or unclear risk).

on asthmatic individuals in this age group that involve aerobic, intermittent, or combined exercise interventions. Our search for adequate methodological quality can partially explain the limitation. The results

of the included studies may have been influenced by small samples,⁴² outcome measurement methods,⁴² intervention duration,⁴² disease severity,⁴³ and lack of certain outcomes.^{43,44}

**Figure 2**

Risk of bias across studies

Conclusion

Asthma leads to biopsychosocial damage. However, exercise can be a good non-pharmacological strategy for reducing the consequences of this pathology. Aerobic exercise led to better PEF and respiratory muscle strength, intermittent exercise led to better FEV₁ and PEF, and both improved functional capacity and quality of life. Combined exercise improved musculoskeletal, VO₂ peak, and cardiopulmonary exercise test results.

Increased methodological rigor and standardized outcome analysis is necessary to increase reproducibility for comparing different types of exercise. New studies are needed to correlate PEF with respiratory muscle strength in intermittent and combined exercise. Further research can help clarify the relationship between exercise and the inflammatory component of asthma, as well as its influence on lung function, functional capacity, quality of life, and reported symptoms.

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Educational interventions for atopic dermatitis in children: what's new?

Intervenções educativas na dermatite atópica em crianças: o que há de novo?

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ABSTRACT

Educating the caregivers of patients with atopic dermatitis may lead to improved quality of life and help minimize disease severity, since the pathology has an immeasurable emotional, social, and financial impact on the patient's families. However, there is no consensus on the ideal scope and frequency for providing educational information about atopic dermatitis and its treatment. The purpose of this integrative literature review was to evaluate studies on the effectiveness of educational interventions for parents and pediatric patients with atopic dermatitis regarding disease severity and quality of life. On August 8, 2022, we searched the PubMed and MEDLINE databases for studies published between 2000 and 2022 in Portuguese or English that included the terms: "atopic dermatitis", "education program", and/or "therapeutic education". A total of 15 articles were included in this review. The studies' methodologies and tools were heterogeneous, impeding comparison of the results. Of the covered topics, skin care and/or basic routines were always included. Although the literature showed that therapeutic education improved disease severity and quality of life for both caregivers and patients, there was no consensus among the authors. Therapeutic education may help increase treatment adherence and prevent complications. However, prospective studies with larger populations are essential for clarifying the ideal scope and frequency of education programs.

Keywords: Atopic dermatitis, child, education.

Introduction

Atopic dermatitis (AD) is a chronic relapsing disease with a prevalence greater than 20% in children.¹ This disease begins in childhood and continues into adulthood in up to 60% of patients.²

RESUMO

A educação dos cuidadores dos pacientes com dermatite atópica (DA) pode possibilitar a melhora da qualidade de vida e minimizar a gravidade da doença, já que esta tem um imensurável impacto emocional, social e financeiro nas famílias dos doentes. Entretanto, não há um consenso de qual o escopo ideal e frequência para a entrega das informações educativas sobre a patologia e terapêutica. Esta é uma revisão integrativa de literatura cujo objetivo foi avaliar estudos sobre a eficácia de intervenções educativas para pais e pacientes pediátricos com DA na gravidade da doença e na qualidade de vida. Foi realizada uma busca nas bases de dados do PubMed e MEDLINE, em acesso realizado em 08/08/2022, incluindo publicações de 2000 a 2022, nos idiomas português e inglês, incluindo os termos: "atopic dermatitis", "education program", "therapeutic education". Foram incluídos 15 artigos nesta revisão. As metodologias dos estudos são diversas e as ferramentas utilizadas pelos pesquisadores também são heterogêneas, o que dificulta a comparação dos resultados. Dos temas abordados, o cuidado da pele e/ou rotinas básicas foram temas sempre incluídos. E, apesar da literatura demonstrar melhora na gravidade da doença e na qualidade de vida dos cuidadores e pacientes com DA após as intervenções, isso não foi um consenso entre os autores. A educação terapêutica pode ser útil para aumentar a adesão ao tratamento e prevenção de complicações; porém, estudos prospectivos com casuística maior são primordiais para esclarecer qual o escopo ideal e frequência para a entrega destas informações.

Descritores: Dermatite atópica, criança, educação.

Therapeutic failure and difficulty in controlling AD result from inability to adhere to long-term therapeutic protocols, which usually involve many medications and need for changes in family routine. Lack of

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information, excessive effort to control the disease, helplessness during periods of disease worsening, and lack of confidence in medical treatment lead to suboptimal management of AD and to increased use of health resources, including alternative therapies,^{3,4} often used without scientific evidence, hoping for a “miraculous cure.”

Education of parents of pediatric patients with AD makes it possible to reduce disease severity, improve quality of life, increase care perception, develop coping strategies, and reduce costs; therefore, it should be implemented in daily clinical practice.⁵ However, there is no consensus on the ideal scope and frequency for providing information about this topic.⁶

The purpose of this integrative literature review was to evaluate studies on the effectiveness of education interventions for parents and pediatric patients with AD regarding disease severity and quality of life.

Data sources

On August 8, 2022, we searched the PubMed and MEDLINE databases for studies published between 2000 and 2022 in Portuguese and English, for theoretical background and contextualization of the theme. Search descriptors were used both in Medical Subject Heading (MeSH) and *Descritores em Ciências da Saúde* (DeCS) databases, and the search was made in sources of published data.

The search strategy included the following terms: “atopic dermatitis,” “education program,” “therapeutic education.” We included empirical research articles that assessed therapeutic education with children and/or adolescents diagnosed with AD and/or their caregivers as interventional strategy. Furthermore, article reference lists were examined to identify relevant eligible papers that could supplement electronic search.

The scientific quality of studies was evaluated based on the following categories of analysis: (1) research objectives; (2) target population and participants' age; (3) study design and use of a control group; (4) follow-up to evaluate the maintenance of intervention results; (5) measuring instruments used; (6) topics covered in the program; and (7) main results found.

A total of 162 citations were initially identified. After reading of the titles, articles not related to the topic and repeated articles were excluded. Subsequently, article abstracts were read. Fifty-six articles were

selected for full reading, according to inclusion and exclusion criteria. After full reading of the articles, we included 2 more articles identified in the reference lists, which have not been found in the initial search. Therefore, a total of 15 articles were included in this review (Figure 1).

Results and discussion

Despite the use of evidence-based guidelines and several therapeutic options, symptoms of AD are not often controlled effectively. The disease has a significant emotional, social, and financial impact on children and their families.⁷ Patients with AD have impaired quality of life, greater chance of developing anxiety and depression, and increased need for health resources.⁷⁻¹⁰

The family of children with chronic diseases is overwhelmed with the care required to control the disease, which makes it difficult to provide appropriate care and comply with recommendations for long periods.¹¹ It has been described that children with AD demand approximately 2 to 3 hours of care daily.¹² Furthermore, skin care of children with AD requires that caregivers be able to perform additional tasks such as following care regimens to minimize flare-ups.^{13,14}

An observational study conducted in France between 2019 and 2020 evaluated the characteristics of parents, children, and adolescents who participated in therapeutic educational programs. A feeling of guilt was observed in parents of patients with AD, resulting either from the thought of having transmitted the disease to their children or exposed them to foods or environmental allergens that parents believe to cause AD, or from the feeling of abandoning their other children who do not have the disease.⁵

Evidence shows that patient's self-care has beneficial therapeutic effects. Problems such as anxiety, frustration, embarrassment, depression, and lifestyle changes can be minimized by means of support groups, family therapies, or individualized follow-up^{7,11,15}; therefore, one of the approaches to promote self-care skills is providing basic information on disease nature and treatment, through support and information services, in addition to providing learning material.¹⁶ A study conducted in Spain to evaluate satisfaction of parents of patients who participated in therapeutic educational groups on AD showed that these parents considered the provided information

useful to achieving efficient control of flare-ups and for improving quality of life.³

However, in clinical practice, it is difficult for health care professionals to properly educate their patients, since many physicians do not have sufficient time to successfully provide all relevant information during the short duration of a medical consultation.¹⁷ Education provided to parents and children during a consultation lasting for 20 to 30 minutes is insufficient,

because both physiopathology and treatment of AD are complex.¹⁸

Initiatives to incorporate digital tools, such as new forms of providing educational information, including videos and online resources, have shown promising results. With technological advances, there has been an increase in the use of mobile technologies and Internet access.⁶ It is possible to deliver alerts and reminders to patients for supporting behavioral

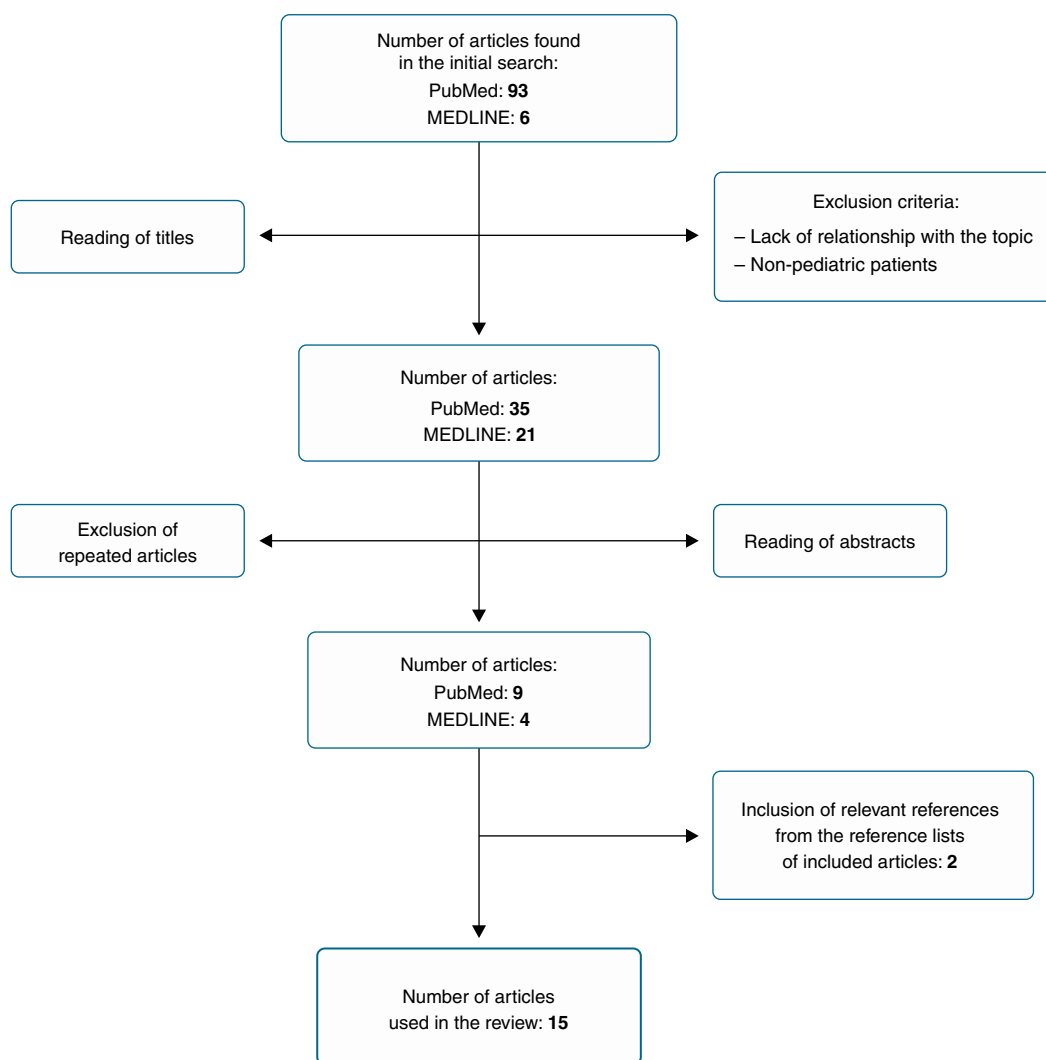


Figure 1
Study selection flowchart

changes via information technology tools, such as cell phone text messages and other wireless technologies.⁶

A systematic review published in 2020 included 13 randomized clinical trials and aimed to summarize evidence about the effectiveness of educational programs for parents of pediatric patients with AD.⁶ The present study added two new publications on the topic. Table 1 summarizes the results of the 15 retrieved articles on educational interventions in pediatric patients with AD.^{4,17-28,30}

The scope of educational interventions varies among the studies. Of the covered topics, skin care and/or basic routines were always included, highlighting the importance of using moisturizers to improve cutaneous barrier function, which is dysfunctional in patients with AD.

In addition to the importance of emollients, the topics “use of corticosteroids” and “corticosteroid phobia” were investigated by some authors.^{27,29} Topical corticosteroids are the mainstay of treatment of AD inflammatory lesions; however, fear of medication use is one of the main reasons for non-adherence and crises, affecting from 60 to 73% of patients.³¹

Therefore, despite lack of consensus on ideal scope and frequency for providing information on AD, it is essential that educational programs discuss topics such as skin care, psychological problems, and use of topical corticosteroids.⁶ Treatment adherence is usually low in patients with chronic diseases, especially in those with dermatosis whose treatment should be topically applied on a regular basis; moreover, family knowledge is crucial to adjust the therapy to each stage of the disease course.³

The methodologies of educational intervention studies in AD were heterogeneous, as well as the tools used by each researcher, impeding comparison of the results.

In six studies, participants attended one therapeutic educational session,^{17-20,22,23} and in two of which written material was provided with information about AD.^{18,22} Five studies included more than one session (with weekly or fortnightly intervals)^{4,21,24,26,29}; in two of which written material was provided to participants.^{24,26} One study used daily text messages²⁸; another one provided a handbook about routine care and AD³⁰; and two used videos on the disease.^{25,27}

The interventions conducted in the retrieved studies focus on secondary prevention of AD, which

would be patients' therapeutic education. However, these studies are limited in terms of educational interventions in primary prevention. A randomized trial with expecting mothers, and later their children, has been ongoing in China since 2020, aiming to evaluate the effect of prenatal educational intervention on the incidence of AD up to 2 years postpartum.³²

Therefore, there is the need for standardized methods to accurately evaluate improvements in self-management of AD.^{6,33,34}

Although the literature showed that therapeutic education improved disease severity and quality of life for both caregivers and patients with AD,^{4,17,18,21-24,26,27,29} there was no consensus among the authors.

Some authors did not find a difference in disease severity or quality of life between intervention and control groups and concluded that the educational intervention may have not been sufficient to change parents' behavior and improve eczema control in children.^{19,20,25,28,30} Furthermore, the relationship between educational interventions and clinical results, including eczema severity, quality of life, and family impact, is not often accurately analyzed in clinical investigations.¹⁷

It is known that designing an educational intervention is a complex activity, because it may have a variety of effects and specific interactions.⁴ Psychological and nutritional factors, as well as a combination of topical and systemic therapies, should also be considered as a strategy to control a disease with an underlying multifactorial physiopathology, such as AD.³⁵

Conclusion

It is common sense that the best approach for treating patients is considering their physical and emotional aspects, in addition to the impact of disease on their personal life and on the family context. Therapeutic education may help increase treatment adherence, and thus its efficacy, as well as prevent complications. Health teams should encourage initiatives based on educational tools and programs, in order to ensure the active engagement of patients in decision-making related to their care. Prospective studies with larger populations are essential for clarifying the ideal scope and frequency of education programs.

Table 1

Summary of articles about educational interventions for parents and pediatric patients with AD regarding disease severity and quality of life

Year/ Ref	Age (years)	Follow-up	Treatment/ control (n)	Treatment	Control	Content	Assessment methods	Result
2002 ¹⁹	0.5-4.0 4.0-16.0	12 weeks	55/42 50/50	Single session	Not specified	Skin care, treatment, basic medical information	CDLQI, IDQOL, DFI	There was no statistically significant improvement in quality of life or eczema
2006 ¹⁷	0.0-16.0	12 weeks	32/29	Single workshop	Standard routine care and written material	Skin care, basic medical information, triggering factors	SCORAD, DFI, CDLQI, IDQOL	Improvement in SCORAD in the intervention group. There was no difference in quality of life between the groups
2006 ⁴	0.25-7.0 8.0-12.0 13.0-18.0	12 months	274/244 102/83 70/50	Group sessions (once a week for 6 weeks)	Standard routine care	Skin care, basic medical information, psychological factors, food allergy, nutrition	SCORAD, POSCORAD, Quality of life questionnaire for parents, JUCKKI/JUCKJU	Improvement in disease severity in all intervention groups compared with control groups
2008 ²⁰	0.0-18.0	1 to 3 months (according to disease severity)	51/55	Single individual session	Not specified	Skin care, treatment, triggering factors, relief of pruritus, lifestyle	SCORAD, CDLQI, IDQOL	There was no difference in disease severity or quality of life between the groups
2008 ²¹	2.0-16.0	24 months	16/16	Fortnightly group sessions for 6 months	Not specified	General overview of the disease, treatment followed by discussion	CDLQI, DFI, McGill pain questionnaire (pruritus)	Improvement in pruritus and quality of life in the intervention group

EASI = Eczema Area and Severity Index, SCORAD = Scoring of Atopic Dermatitis, CDLQI = Children's Dermatology Life Quality Index, IDQOL = Infants' Dermatology Quality of Life Index, DLQI = Dermatology Life Quality Index, PO-SCORAD = Patient Oriented SCORAD, FDLQI = Family Dermatitis Life Quality Index, STAI = State Trait Anxiety Inventory, PPS = Perceived Stress Scale, DLQI = Dermatitis Life Quality Index, QOLAD = Quality of Life Index for Atopic Dermatitis, BDI = Beck Depression Inventory, EQ-5D = EuroQol 5-Dimension, POEM = Patient-Oriented Eczema Measure, HADS-D = Hospital Anxiety and Depression Score, DFI = Dermatitis Family Impact, RC = randomized control trial, JUCKKI/JUCKJU = Itching cognitions questionnaires, AD = atopic dermatitis.

Table 1 (continuation)
Summary of articles about educational interventions for parents and pediatric patients with AD regarding disease severity and quality of life

Year/ Ref	Age (years)	Follow-up	Treatment/ control (n)	Treatment	Control	Content	Assessment methods	Result
2009 ²²	< 16	4 weeks	49/50	Single individual session with written material	Standard routine care	Basic medical information, triggering factors, treatment	SCORAD	Improvement in SCORAD and greater adherence in the intervention group
2010 ²³	< 4 4-16	12 months	37/34 35/35	Single group or individual session in nursing consultations	Standard routine medical care	Skin care, basic medical information, allergies, practical and theoretical sessions	SCORAD, CDLQI, IDQOL, DFI, Satisfaction program	Improvement in SCORAD and quality of life in both groups
2013 ²⁴	0.5-6.0	6 months	29/30	Two-day program with lectures and written material	Standard routine care and written material	Skin care, basic medical information, treatment, adverse effects, triggering factors	SCORAD, POSCORAD, questionnaire on pruritus, insomnia, amount of corticosteroid used, DFI	Improvement in disease severity and amount of corticosteroid used in the intervention group
2016 ¹⁸	0.5-12.0	2 months	64/64	Single lecture with written material	Routine standard care	Skin care, diagnosis, treatment, triggering factors, diet, quality of life	SCORAD, FDLQI, STAI, PPS	Improvement in disease severity, anxiety, pruritus, insomnia, and stress in the intervention group
2017 ²⁵	0.3-4.3	1 week	10/11	Video with tailored leaflet	Same video without leaflet	Skin care, ideal amount of moisturizers	Use of moisturizers, EASI	There was no difference in AD severity between the groups

EASI = Eczema Area and Severity Index, SCORAD = Scoring of Atopic Dermatitis, CDLQI = Children's Dermatology Life Quality Index, IDQOL = Infants' Dermatology Quality of Life Index, DLQI = Dermatology Life Quality Index, PO-SCORAD = Patient Oriented SCORAD, FDLQI = Family Dermatitis Life Quality Index, STAI = State Trait Anxiety Inventory, PPS = Perceived Stress Scale, DLQI = Dermatitis Life Quality Index, QoLAD = Quality of Life Index for Atopic Dermatitis, BDI = Beck Depression Inventory, EQ-5D = EuroQol 5-Dimension, POEM = Patient-Oriented Eczema Measure, HADS-D = Hospital Anxiety and Depression Score, DFI = Dermatitis Family Impact, RC = randomized control trial, JUCKKI/JUCKJU = Itching cognitions questionnaires, AD = atopic dermatitis.

Table 1 (continuation)
Summary of articles about educational interventions for parents and pediatric patients with AD regarding disease severity and quality of life

Year/ Ref	Age (years)	Follow-up	Treatment/ control (n)	Treatment	Control	Content	Assessment methods	Result
2018 ²⁶	4.0-14.0	6 months	293/249	Four sessions (once a week), videos and printed material to be used at home	Not specified	Skin care, long-term management, food allergy, psychological factors	SCORAD, IDQOL, CDLQI, Questionnaire to assess knowledge	Improvement in disease severity, quality of life, and use of emollient in the intervention group
2018 ²⁷	0-16	6 weeks	5/5	Video - AD	Video – placebo topic	Corticosteroid phobia, treatment adherence	EASI	Improvement in disease severity in the intervention group. No difference in corticosteroid phobia and adherence
2018 ²⁸	0.3-3.8	42 days or up to follow-up visit	14/16	Daily educational text messages	Standard routine care	Skin care, triggering factors	EASI	There was no difference in AD severity between the groups
	6-16	12 to 18 months	14/0	Six individual sessions + seven group sessions	Not specified	Understanding on AD, corticosteroids, emollients, allergies, treatment, pruritus, social isolation	SCORAD, school absenteeism	Improvement in SCORAD and school absenteeism for all patients
2021 ³⁰	0.0-16	3 months	91/84	Handbook	Standard routine care	Understanding on AD, treatment, psychological factors	EASI, IDQOL, CDLQI, DFI, POEM, Questionnaire on parenting self-efficacy and management of tasks related to AD improvement	No difference in symptom between the groups. The handbook improved family's confidence in their management skills

EASI = Eczema Area and Severity Index, SCORAD = Scoring of Atopic Dermatitis, CDLQI = Children's Dermatology Life Quality Index, IDQOL = Infants' Dermatology Quality of Life Index, DLQI = Dermatology Life Quality Index, PO-SCORAD = Patient Oriented SCORAD, FDLQI = Family Dermatitis Life Quality Index, STAI = State Trait Anxiety Inventory, PPS = Perceived Stress Scale, DLQI = Dermatitis Life Quality Index, QoLAD = Quality of Life Index for Atopic Dermatitis, BDI = Beck Depression Inventory, EQ-5D = EuroQol 5-Dimension, POEM = Patient-Oriented Eczema Measure, HADS-D = Hospital Anxiety and Depression Score, DFI = Dermatitis Family Impact, RC = randomized control trial, JUCKKI/JUCKJU = Itching cognitions questionnaires, AD = atopic dermatitis.

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Mast cells and mast cell activation syndrome: what's new?

Mastócitos e síndrome de ativação mastocitária: o que há de novo?

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ABSTRACT

Mast cells are the main effector cells of acute allergic response, also playing an important role in angiogenesis, immune tolerance, regulation of fibrinolysis, neuronal regeneration, and osteoclastogenesis. They are generally located in the skin and mucous membranes of the intestines and lungs, where they perform a "sentinel" function. Mast cell activation syndrome is characterized by recurrent clinical manifestations resulting from the release of mast cell mediators. This complex entity, which involves a spectrum of associated symptoms, is a diagnostic and therapeutic challenge. In this article we overview of the structure and function of mast cells, in addition to the diagnostic criteria and therapeutic approaches to mast cell activation syndrome.

Keywords: Mast cells, mastocytosis, mast cell activation syndrome.

RESUMO

Os mastócitos são as principais células efetoras da resposta alérgica aguda, desempenhando também um papel importante na angiogênese, tolerância imunológica, regulação da fibrinólise, regeneração neuronal e osteoclastogênese. Localizam-se maioritariamente na pele e nas mucosas do intestino e pulmões, onde exercem uma função "sentinela". As síndromes de ativação mastocitária são caracterizadas pela ocorrência de episódios recorrentes de manifestações clínicas resultantes da libertação de mediadores mastocitários. Esta constitui-se como entidade complexa com um espectro de sintomas associados, representando um desafio diagnóstico e terapêutico. Nesta revisão, os autores pretendem apresentar uma visão geral sobre a estrutura e função dos mastócitos e sobre os critérios diagnósticos e abordagem terapêutica da síndrome de ativação mastocitária.

Descritores: Mastócitos, mastocitose, síndrome de ativação mastocitária.

Introduction

Mast cells are connective tissue cells originating from hematopoietic cells located in bone marrow, which may play important roles in host defense to parasitic infections and in allergic reactions. Mast cells are ubiquitously located in the connective tissue,

skin, and around blood vessels, but are also located in mucous surfaces of intestine and lungs.¹ Mast cells are very important cells in inflammatory response, since their activation promotes the release a wide variety of pro-inflammatory mediators, of which the following

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stand out: histamine, tryptase, cytokines such as interleukins IL-4 and IL-13, and tumor necrosis factor (TNF)- α , or even lipid mediators, such as leukotrienes, platelet-activating factor, and prostaglandins.¹⁻³

In recent years, evidence has emerged that mast cell activation and proliferation may occur in some individuals in a pathological way and can be triggered by several stimuli, such as stress, heat, or cold.²

The term mast cell activation syndrome (MCAS) refers to a heterogeneous set of diseases clinically characterized by the occurrence of recurrent episodes of clinical manifestations resulting from the release of mast cell mediators. Given the presence of clonality, MCAS may be classified into clonal MCAS (CMCAS) and non-clonal MCAS (NCMCAS).¹⁻⁴

CMCAS include monoclonal MCAS (MMCAS), cutaneous mastocytosis (CM), and systemic mastocytosis (SM), which present with clinical manifestations resulting from mast cell activation.^{5,6} Conversely, NCMCAS may be grouped into secondary (e.g., associated with IgE-mediated hypersensitivity) or idiopathic.¹⁻³

Several diagnostic criteria have been suggested so far, often lacking validation.⁴ This is an urgent and relevant need, since a growing number of individuals have been misdiagnosed with MCAS, based on unspecific criteria. Therefore, this article aims to critically address currently literature related to MCAS, comparing the criteria presented by two groups of authors more devoted to this area.

Methods

The authors conducted a literature search on the PubMed database covering up to May 31, 2022, and using the following terms: *mast cell*, *mastocytosis*, *anaphylaxis*, combined with the terms *activation syndrome*, *tryptase*, *consensus*. Articles addressing issues related to MCAS published in the last 10 years were used, focusing especially on articles published by authors from reference centers and on consensus publications. Occasionally, we used articles published more than 10 years ago that are still currently relevant.

Importance of mast cells in inflammatory response

Mast cells are the main effector cells of acute allergic response. However, they can also play other

roles in angiogenesis, immunomodulation, regulation of fibrinolytic mechanisms, regeneration of nervous fibers, and promotion of osteoclastic differentiation.⁷

These cells originate from bone marrow, being derived from the hematopoietic lineage (CD34+/CD117+ progenitor cells). After traveling briefly through bloodstream, they differentiate in the connective tissue in general (including mucosae), where they acquire their specific phenotype, in the presence of local growth factors such as IL-9, IL-10, IL-3, IL-4, IL-33, CXCL12, neuronal growth factor (NGF), and transforming growth factor (TGF)- β .^{1,3,8} In structural terms, they present with granules containing histamine, tryptase, heparin, chondroitin, sulphates, and other glycosaminoglycans.⁸

These cells may be classified in different ways, based specifically on granular concentration of serine, tryptase and chymase proteases.^{3,9} Mast cells whose granules contain mainly tryptase (MCT) are predominantly located in the alveolar septa and in small intestine mucosa, whereas mast cells that contain both proteases (MCTC) may be found in skin, layers of small intestinal submucosa, and also in the connective tissue.^{3,9} MCT, which are found mainly in mucosae, interact closely with lymphocytes T, namely Th2 cells.⁵ Globally, they are located in tissues, skin, blood vessels, and mucosae.³

Mast cell membrane receptors

Mast cells have a diversity of membrane and cytoplasmic receptors, which allows them to be activated by several stimuli, such as anaphylatoxins (C3a and C5a), IgG, medicines, insect venoms, physical stimuli (variations in pressure and temperature), neuropeptides, cytokines (IL-4R α , IL-5R α , IFN- γ R α , and ST2), and vascular endothelial growth factors (VEGFR1 and VEGFR2).⁷ They also express receptors for several ligands, such as toll-like receptors (TLR), which are activated by pathogen-associated molecular patterns (PAMPs), being thus involved in responses to bacteria, parasites, and viruses.¹⁰

Allergic activation (mediated by IgE) promotes mast cell degranulation, with release of preformed molecules such as histamine, serotonin, proteases (tryptase, chymase, carboxypeptidase), and tumor necrosis factor (TNF), as well as *de novo* secretion of numerous vasoactive and pro-inflammatory mediators, including leukotrienes, prostaglandins, PAF, cytokines (IL-6, IL-9, IL-13), and chemokines (CXCL8, CCL2, CCL5).¹¹⁻¹³

In addition to IgE-dependent reactions, in which high-affinity IgE receptors (FC ϵ RI) of mast cells are extremely relevant (non-covalent tetrameric complexes that, together with IgE, will attach to the antigen and trigger mast cell activation), there are also non-IgE-mediated reactions, in which activation of the complement system and respective mast cell degranulation occur.³ In addition to these two types of reactions, there also are non-immunologic reaction, in which there is the direct mast cell degranulation mediated by MAS-related G Protein-Coupled Receptor-X2 (MRGPRX2).³

All mast cells express KIT receptor (CD117), whereas only some types of mast cells (e.g., those located in the skin or in synovial fluid) express MRGPRX2, which is activated by neuropeptides such as substance P.⁷

Maturation and regulation of immunological and non-immunological cells

Mast cells play very different roles in maturation and activation/regulation of several cells, namely dendritic cells or lymphocytes T and B, through the release of numerous mediators (histamine, prostaglandins, leukotrienes, tryptase, chymase, carboxypeptidase, and numerous interleukins) present within mast cells.¹⁴

Furthermore, mast cells can activate macrophages (through histamine, IL-13, IL-6, PAF and PGD₂), dendritic cells (after release of histamine, PGE₂, PGD₂, VEGF-C and IL-13), and innate lymphoid cells (ILCs), via IL-1 β , IL-9, PGD₂, and LTD₄. Conversely, mast cells stimulate NK cells through the release of histamine and heparin, and also T cells, through the release of histamine, LTC₄, LTD₄ and TNF- α .¹⁵

Endothelial cells also undergo mast cell regulation, through the release of mediators such as histamine, LTC₄, LTD₄, PGD₂, PAF, VEGF-A, IL-13, and IL-1 β . The regulatory effect is also exerted on bronchial epithelial cells, smooth muscle cells, fibroblasts, or lymphatic endothelial cells, among others, with the release of VEGF-C and VEGF-D to promote angiogenesis thorough endothelial cell proliferation.¹⁵

Due to their distribution, versatility response to multiple stimuli, and variety of molecules they release, mast cells play the role not only of “sentinel” of the immune system but also of maintaining homeostasis. Moreover, they are involved in several physiological and pathological processes, in the context of innate immunity,

autoimmunity, neuroinflammation, immunomodulation, and antimicrobial activity.^{10,11,16-20}

Types of mast cell activation

Mast cells are located in tissues that interact continuously with exterior. Additionally, they are stimulated by a vast number of different triggers, including bacteria, medicines, foods, and physical agents that promote the secretion of mediators.²¹ It is possible to observe that there are two main modes of mast cell degranulation: the first one, named anaphylactic degranulation, is a rapid and immediate event that occurs after IgE activation, whereas the second one, piecemeal degranulation, is slower.²² Some examples of mast cell degranulation triggers include acetylcholine, complement factors, medicines (some antibiotics and anesthetics), peptides such as endorphin, endothelin, and somatostatin, and physical stimuli such as heat, cold, pressure, stress, and vibration. Mast cells also respond to non-allergic activators through mediators that can be released without degranulation. Finally, corticotropin-releasing hormone (CRH), cytokines, and some microorganisms have been identified as mast cell triggers without degranulation.^{21,22}

Mast cell activation syndrome

MCAS is a complex entity characterized by the occurrence of acute severe recurrent episodes whose heterogeneous symptoms involve at least two organ systems and present multiple triggering agents, posing important diagnostic and therapeutic challenges. There are two types of MCAS to consider. Clonal (or primary) MCAS is characterized by the presence of clonal markers, such as *KIT*^{D816V} mutation, abnormal expression of CD25 or CD2 by mast cells in bone marrow, or atypical morphology (e.g., fusiform, binucleated).^{1,2} Non-clonal MCAS is subdivided into two types, the first of which is caused by a known stimulus (secondary MCAS), whereas in the second type mast cell degranulation is caused by an unknown stimulus (idiopathic MCAS).²³

MCAS is a disease of complex etiology whose diagnosis is usually late and challenging.^{1,2,21} Patients may present with signs and symptoms that mimics cardiovascular, gastrointestinal, respiratory, or cutaneous diseases, or compatible with allergic reactions and recurrent anaphylaxis episodes, which may be IgE-mediated or not.²¹

Triggering factors and symptoms

Several stimuli can trigger mast cell degranulation, including allergens (similar in frequency to the general population), bacteria, viruses, toxoids, food, alcohol, medicines (non-steroidal anti-inflammatory drugs [NSAIDs], opiates, and contrast media, among others), or even physical agents such as pressure, friction, cold, or heat.²¹ Stress also seems to play an important role, as well as hormone changes.²⁴⁻²⁶

Typical manifestations of MCAS can include acute urticaria, flushing, pruritus, abdominal pain, diarrhea, shortness of breath, and hypotension. None of these manifestations are absolutely specific, but the simultaneous development of a set of recurrent signs and symptoms related to at least two organs or body systems are highly suggestive for MCAS.^{4,27}

The main clinical manifestations of the syndrome are described in Table 1.

In addition to the aforementioned clinical manifestations, objective laboratory evidence of mast cell involvement, confirming a substantial transient increase in mast cell mediators and control of symptoms with drug therapy is required for the diagnosis of MCAS to be established.⁴

There is another myriad of non-specific symptoms/diseases that are not specific for this disease (Table 2).

Differential diagnoses

These same symptoms may be present in other clinical entities, including some cardiovascular, endocrine, cutaneous, gastrointestinal, and neuropsychiatric diseases.

In general, MCAS is a syndrome defined by a severe, recurrent systemic reaction (often in the form of anaphylaxis), resulting from a clinically significant release of vasoactive and pro-inflammatory mast cell mediators.^{1,4} When there are no signs of anaphylaxis, MCAS is much less likely to be the correct diagnosis. For less severe or localized forms of mast cell activation (e.g., limited to skin or respiratory tract) not meeting MCAS criteria, further research is needed to evaluate these patients.

In recent years, a specific type of NCMCAS has been described, named hereditary alpha-tryptasemia (HaT), which is an autosomal dominant inherited disease with still unknown penetrance, caused by increased number of *TPSAB1* gene copies.⁴ With a prevalence ranging from 5% to 6% of the general population, HaT results in elevated baseline tryptase, often greater than 10 ng/mL.⁴ Up to two thirds of patients with this condition may have mild symptoms or being completely asymptomatic.⁴ The other patients may present with dysautonomia with postural orthostatic hypotension, joint hypermobility, vibratory urticaria, irritable bowel syndrome, chronic musculoskeletal pain, and a myriad of manifestations compatible with respiratory and/or cutaneous allergic disease, such as shortness of breath, cough, wheezing, anterior/posterior rhinorrhea, or sneezing.⁴

Some authors recommend evaluating the presence of the gene *TPSAB1* gene to determine the presence of concomitant HaT in these patients. However, investigation for the presence of HaT is mandatory in individuals with mastocytosis or allergic to hymenoptera venom.^{4,21,25} The exact percentage of patients with clonal and idiopathic MCAS who currently have HaT is unknown.⁴

Table 1
Mains organ systems involved in MCAS and main clinical manifestations of the disease

Skin	Flushing, urticaria, pruritus, angioedema
Cardiovascular	Syncope/presyncope, hypotension
Gastrointestinal	Diarrhea, vomiting, abdominal pain, gastroesophageal reflux
Respiratory	Nasal/conjunctival congestion and pruritus, sneezing, wheezing, shortness of breath, laryngeal edema, hypoxia

MCAS = mast cell activation syndrome.
Adapted from Gülen T et al. (4)

Table 2

Symptoms and diseases not diagnostic for MCAS

NOT diagnostic symptoms/
diseases of MCAS

Fatigue, fibromyalgia-like pain, chronic back pain, edema, dermatographism, alopecia, warts, tinnitus, adenopathy, weight changes, hypo/hyperthyroidism, metabolic syndrome, electrolytic changes, type 2 diabetes mellitus, gastroenterology disease (constipation, gastroesophageal reflux, irritable bowel syndrome, inflammatory bowel disease, celiac disease), neuropsychiatric disease (headache, attention deficit /hyperactivity disorder, anxiety, depression, posttraumatic syndrome, mood disturbances, restless leg syndrome, schizophrenia), essential hypertension, pulmonary hypertension, atherosclerosis, dysautonomia syndrome (POTS), joint hypermobility (hEDS), chronic kidney disease, prostatitis, endometriosis, polycystic ovary syndrome, autoimmune diseases, multiple food/chemical sensitivity syndrome, malignancies, anemia, polycythemia, quantitative changes in antibodies, nonspecific peripheral blood mutations

MCAS = mast cell activation syndrome.
Adapted from Gülen T et al.⁴.

Diagnostic criteria

Diagnostic criteria for MCAS have been proposed by the European Competence Network on Mastocytosis (ECNM)^{4,25,28} and by Molderings et al.^{2,29}

According to the ECNM, there are three diagnostic criteria for suspected cases of MCAS: recurrent severe episodes with typical systemic symptoms of mast cell activation involving two or more organs; increase of 20% in serum tryptase level above individual's baseline plus 2 ng/mL within 4 hours after a symptomatic crisis (e.g., an increase of 10 ng/mL for values above 14 ng/mL, or of 30 ng/mL for values above 38 ng/mL); favorable therapeutic response to antimediation therapy (e.g., antihistamine H1 and H2, mast cell membrane stabilizing agents, antileukotrienes) (Table 3).^{4,25}

MCAS can be classified into 3 subtypes: 1) primary (or monoclonal) MCAS with *KIT*^{D816V} mutation, with or without diagnosis of mastocytosis; 2) MCAS secondary to IgE-dependent allergy, hypersensitivity reaction, or immunological disease likely to induce mast cell activation (e.g., Hashimoto thyroiditis); 3) idiopathic MCAS, in which no KIT mutation or other underlying condition could be detected.^{4,25} Patients who simultaneously have IgE-dependent hypersensitivity and mast cell clonality can be classified as presenting with mixed MCAS.⁴

Molderings et al. proposed that the diagnosis of MCAS involves major and minor criteria (Table 4). Diagnosis can be established up demonstration of both major criteria, or alternatively, the second major criterion combined with at least one minor criterion.²⁹

According to Molderings et al., this disease may affect up to 17% of the general population², which the authors of this article believe to represent an overdiagnosis of the disease, since the suggested diagnostic markers show low sensitivity. Conversely, according to the ECNM, when including an elevation of mediators relatively specific for mast cells, it would also be important to define their respective thresholds. The ECNM believes that patients with typical symptoms of MCAS without confirmed acute elevation of any validated biomarker of mast cell activation should not be given a diagnosis of MCAS. An argument against using such markers is the limited access to these tests. Furthermore, MCAS patients may experience certain symptoms almost chronically, so that a truly asymptomatic serum baseline tryptase that allow for comparison between episodes is difficult to obtain.⁴

However, many reactive inflammatory states and neoplasms, as well as individuals with no specific symptoms, may present with an increase in local

Table 3
Proposed diagnostic criteria for MCAS according to the European Competence Network on Mastocytosis (ECNM)^{4,25,27}

Typical MCAS-related clinical symptoms (see Table 1)
Event-related increase > 20% in baseline serum tryptase plus 2 ng/mL within 4 hours after a symptomatic crisis
Improvement of symptoms with the use of H1 antihistamine, other stabilizing agents, or drugs directed to mast cell mediators

MCAS = mast cell activation syndrome.

mast cells without MCAS and without filling criteria for this disease.⁴

General approach

The approach of MCAS involves the implementation of prophylactic and therapeutic measures for its control. After ruling out other diseases that can mimic MCAS, it is essential for patients to know and avoid agents likely to trigger their episodes of MCAS.

However, similar that what occurs with mastocytosis, generalized avoidance of these potential stimuli is not recommended.³⁰

Nonetheless, there is a considerable variety of stimuli, such as exposure to extreme temperatures or temperature variations, excessive solar exposure, emotional stress, sleep deprivation, physical stimuli, alcohol, and susceptible medicines. NSAIDs, opioids, anesthetic agents, and iodide contrast media may also trigger episodes of MCAS.^{31,24}

Table 4
Proposed diagnostic criteria for MCAS according to Molderings et al.^{2,29}

Major criteria	Minor criteria
Presence of multifocal mast cell aggregates in bone marrow (BM) or extracutaneous organs (ECO)	Abnormal morphology in > 25% of mast cells in BM of ECO
Clinical history compatible with recurrent/chronic release of mediators due to increased mast cell activity	Mast cell expression of CD2 and/or CD25.
	Presence of mast cell genetic mutations (<i>KIT</i> ^{D816V} mutation)
	Values above normal for mast cell mediators (serum tryptase, chromogranin A, plasma heparin, plasma histamine, urinary N –methyl-histamine, leukotrienes B4, C4, D4, and E4, PGD2, 11-beta-PGF2α)
	Symptomatic response to mast cell activation inhibitors

MCAS = mast cell activation syndrome.

Premedication protocols are usually recommended for the treatment of mastocytosis. Despite the lack of similar protocols for MCAS, in clinical practice patients with this disease follow premedication protocols in certain conditions, such as before invasive procedures (both surgical and diagnostic), radiological tests with contrast, dental procedures, and vaccination. The protocols usually include H1 and H2 antihistamines, leukotriene antagonists, benzodiazepines, and prednisolone, depending on the case.^{1,30}

It is worth mentioning that the level of evidence of these protocols is low and based on the opinion of some specialists; moreover, any therapeutic protocol should be carefully adapted to the patient and reviewed by the medical team before administration.³⁰

Pharmacological treatment

Pharmacological control depends on the use of different agents, either isolated or combined, according to the intensity, severity, and type of symptoms of each patient. Second generation antihistamine (anti-H1) are the main pharmacological agents to be used in these situations. When necessary, their usual dose can be quadrupled, with no significant secondary effects. There are reports of patients who need to take them on a chronic basis.^{1,25}

There are few studies to support the choice and the dose of H1 antihistamines. Desloratadine and ketotifen showed mast cell stabilization properties.³⁵ Rupatadine has an antagonist effect of PAF receptor, a mediator involved in episodic hypotension and flushing, and was associated with improved quality of life and symptoms in patients with mastocytosis and symptoms of MCAS.^{32,33} Both rupatadine and levocetirizine, at a lower extent, show the inhibiting effect of PAF in mast cell degranulation, which is not observed with desloratadine.³¹⁻³⁵

H2 receptor antagonists, such as famotidine and cimetidine, are particularly useful in dyspeptic gastrointestinal symptoms, abdominal pain, diarrhea, and severe recurrent episodes.²⁸

Leukotriene receptor antagonists, such as montelukast, has proven to be useful in controlling respiratory, cutaneous, and gastrointestinal symptoms.^{1,28,36}

Acetylsalicylic acid may help control flushing and hypotension in selected cases with known tolerance to the drug. In the past it was used in the treatment of cases of anaphylaxis in patients with SM, but the doses required to block mast cell activation are high and not

always tolerated.^{27,36} A study reported that celecoxib could be considered an option for intractable diarrhea in mastocytosis patients.³⁶ Systemic corticosteroids are a resource reserved for individuals with refractory symptoms.²⁸

Therapy with anti-IgE monoclonal antibody, omalizumab, has shown to be effective and with a good safety profile in patients with secondary MCAS who have underlying IgE-dependent allergy, especially anaphylaxis refractory to conventional therapy, as well as in those with primary and idiopathic MCAS.³⁷ A French study including 55 patients diagnosed with MCAS and persisting symptoms treated with omalizumab for 3 years showed an overall best response rate of 78% of patients (maintained for at least 3 months in 77% of them), and a final overall response rate of 58% in the last follow-up.³⁷

Patients with reactions compatible with anaphylaxis should use self-injectable adrenaline devices, two of them if necessary. These patients are prone to develop the most severe, potentially fatal, forms of anaphylaxis, especially those with allergy to insect (hymenoptera) venom.^{30,38}

With regard to hymenoptera venom-induced (IgE-dependent) anaphylaxis, i.e., secondary MCAS in this case, it is recommended to initiate venom immunotherapy (VIT) specific to the concerned venom, which should be maintained throughout life, since severe reactions persist if treatment is interrupted.³⁹

VIT has proved to be effective in SM patients who have an IgE-mediated reaction, although there is a known higher risk of adverse reactions in patients with SM undergoing treatment with VIT compared to the general population.³⁹ Therefore, omalizumab may be useful in reducing associated adverse effects and facilitates building up to the VIT maintenance dose.³⁹

In general, vaccination against COVID-19 is recommended in individuals with MCAS, as well as in individuals with mastocytosis. The only exception to consider is when there is suspected or known allergy to a one of the vaccine components. Patients will need to receive premedication according to their individual risk and undergo a postvaccination observation period, similarly to the general population.⁴⁰

Final considerations

MCAS is a complex entity with unknown prevalence⁴¹ characterized by the occurrence of acute

severe recurrent episodes whose heterogeneous symptoms involve at least two organ systems, presenting multiple triggering agents and posing important diagnostic and therapeutic challenges.

Several differential diagnoses should be carefully evaluated before diagnosing MCAS, such as HaT. An incorrect diagnosis of MCAS may affect patients, delaying appropriate treatment and thus compromising their quality of life and prognosis. In most cases, elevation of serum tryptase during the events confirms mast cell involvement.

Currently two models have been proposed for the diagnosis of MCAS. The first one, established by the ECNM, presents more robust and specific criteria. The other model, proposed by Molderings et al., presents more comprehensive criteria that have not been validated in the specific context of MCAS, leading to a serious risk of overdiagnosing the disease. Nowadays, it has been observed that many patients diagnosed with MCAS do not present confirmed involvement of mast cell activation.

Recent findings have suggested this disease had been identified in a significant number of COVID-19 patients. The scientific community is concerned these patients are actually delaying their true diagnosis, according to the criteria established in the Vienna consensus by the ECNM.

The therapeutic approach of MCAS involves the use of second generation H1 antihistamines, H2 receptor antagonists, montelukast, corticoids, omalizumab (anti-IgE), and mast-cell targeted therapies. Symptomatic control also depends on the identification and cautious avoidance of triggers.

Initial therapy may be implemented before definite diagnosis of MCAS, with response being a useful factor to support diagnosis. According to the type and severity of the syndrome, it may be necessary to combine therapies, including anti-IgE therapy and targeted therapy.

The diagnosis of MCAS is still complex, multidisciplinary, and demanding. In the future, it will be important to discover new biomarkers that allow for physicians to clearly distinguish MCAS from other mimicking diseases.

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Translation and validation of the Drug Hypersensitivity Quality of Life Questionnaire (DrHy-Q) to Brazilian Portuguese

Tradução e validação do Questionário de Qualidade de Vida em Hipersensibilidade a Drogas (DrHy-Q) para a língua portuguesa (cultura brasileira, DrHy-Qb)

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ABSTRACT

Introduction: Drug hypersensitivity is a clinical condition that can impair health-related quality of life. Originally developed in Italian, the Drug Hypersensitivity Quality of Life Questionnaire (DrHy-Q) measures the impact of drug hypersensitivity on quality of life. The objective of this study was to translate, cross-culturally adapt, and validate the DrHy-Q to Brazilian Portuguese (DrHy-Qb). To be successful, the DrHy-Qb must be internally consistent, maintain construct validity, and be reproducible as an assessment tool for quality of life in Brazilian patients with drug hypersensitivity. **Methods:** Translation and back-translation were performed by 3 bilingual translators, followed by a pre-test and a final version. The final version, the DrHy-Qb, and the 36-Item Medical Outcomes Study Short-Form General Health Survey (SF-36) were administered to 84 patients (69% female, 40.3±15.2 years) from a specialized outpatient clinic. Factorial analysis included Pearson's correlation coefficient to validate the construct, Cronbach's alpha to assess the internal consistency, and intraclass correlation coefficient to determine reproducibility. **Results:** Statistical analysis showed excellent internal consistency ($\alpha = 0.936$) and reproducibility ($r: 0.984$; 95% CI: 0.963-0.993; $p < 0.001$). The correlation between the DrHy-Qb and the SF-36 was moderate and negative ($r: -0.394$; $p < 0.01$). **Conclusions:** This study showed that the DrHy-Qb was successfully translated, adapted, and validated into Brazilian Portuguese, and can be used to assess quality of life in patients with drug hypersensitivity.

Keywords: Drug hypersensitivity, surveys and questionnaires, quality of life, language, validation study.

RESUMO

Introdução: A hipersensibilidade a fármacos é uma condição clínica debilitante, acompanhada de experiência emocional intensa e pode afetar a qualidade de vida relacionada à saúde (QVRS). A repercussão das reações de hipersensibilidade a drogas (RHD) na qualidade de vida (QV) pode ser verificada pela utilização de questionário específico, o *Drug Hypersensitivity Quality of Life Questionnaire* (DrHy-Q), desenvolvido originalmente na língua italiana. O objetivo foi traduzir, adaptar transculturalmente e validar a versão do DrHy-Q para a língua portuguesa (cultura brasileira, DrHy-Qb), verificando a consistência interna, validação de constructo e reprodutibilidade do DrHy-Qb, como instrumento específico de avaliação da QV nos pacientes brasileiros com hipersensibilidade a fármacos. **Métodos:** A adaptação do questionário consistiu na tradução e retrotradução realizadas de forma independente por três tradutores bilíngues, seguidas por pré-teste. A versão final, DrHy-Qb juntamente com o questionário de qualidade de vida resumido (SF-36), foi respondido por 84 pacientes (69% feminino, 40,3±15,2 anos) acompanhados em ambulatório especializado. Na análise fatorial, a validação de constructo foi realizada pelo cálculo do coeficiente de correlação de Pearson, de consistência interna pelo coeficiente alfa de Cronbach, e da reprodutibilidade pelo coeficiente de correlação intraclass. **Resultados:** A análise estatística evidenciou consistência interna ($\alpha = 0,936$) e reprodutibilidade ($r: 0,984$; IC95% = 0,963-0,993; $p < 0,001$) excelentes. A correlação entre o DrHy-Qb e o SF-36 total foi negativa e moderada ($r = -0,394$; $p < 0,01$). **Conclusões:** O DrHy-Qb foi adequadamente traduzido, adaptado e validado para a cultura brasileira, podendo ser útil na avaliação da qualidade de vida dos pacientes com hipersensibilidade a fármacos.

Descritores: Hipersensibilidade a drogas, inquéritos e questionários, qualidade de vida, idioma, estudo de validação.

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Introduction

Drug hypersensitivity reactions (DHRs) represent up to 15% of all adverse reactions to drugs.¹ Depending on the mechanism, these reactions can trigger a variety of clinical manifestations such as urticaria, angioedema, bronchospasm, anaphylaxis, exanthems, and other signs and symptoms. The skin is affected in more than 90% of the cases.²

The relationship between clinical manifestations and suspected drugs is frequently hard to be determined, hindering adequate patient follow-up. The absence of an accurate etiological diagnosis interferes with the choice of specific treatments, which may not be as effective when compared to first-line options.³

In any case, DHRs significantly influence clinical practice and are a frequent cause of increased hospitalization times, changes to medical prescriptions, and a consequent socioeconomic impact.²

In clinical practice, from the patients' point of view, a history of DHRs is an experience that comes along with an intense emotional load, such as fear, anxiety, somatization, and insecurity when faced with the need to use any drugs that may negatively affect their life and compromise their everyday functioning.⁴ The assessment of health-related quality of life (HRQoL) is important for measuring the impact of diseases on patients' daily lives.⁵

The repercussion of DHRs in quality of life (QoL) can be verified by using a specific questionnaire, the Drug Hypersensitivity Quality of Life Questionnaire (DrHy-Q), which was originally developed in the Italian language. This is a single-domain instrument comprising 15 items that met standards of construct validity, internal consistency, and reliability; it is thus appropriate for use alone or in combination with other questionnaires with the aim of reporting the emotional experiences of patients with drug hypersensitivity.⁶

DrHy-Q was also translated and validated to the Turkish, Dutch, Spanish, Thai, and Portuguese (Portugal) languages.⁷⁻¹¹

The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) has a Brazilian version; this is a generic health assessment instrument comprising 36 questions of easy implementation and understanding that can be applied to the general population.¹²

There are no Brazilian studies assessing the QoL of patients with drug hypersensitivity, nor a validated version of the DrHy-Q in Portuguese (Brazilian culture).

The development of a specific tool would be time-consuming and onerous and would hamper the comparison of the obtained results with those observed in other countries. Hence the importance of the cross-cultural adaptation of the DrHy-Q, a valid and reliable instrument.

This study aimed to translate, adapt, and validate the original version of the DrHy-Q for the Portuguese language (Brazilian culture, DrHy-Qb) and verify its validity, reliability, and reproducibility.

Methods

This is a cross-sectional study of a population followed-up between November 2017 and December 2019 in a specialized outpatient clinic. During this period, the clinic received 174 patients over 18 years old with a suspected history of DHRs.

For adapting and validating the DrHy-Qb, we used a convenience sample of 94 patients with a probable history of DHRs.¹³ According to the causality scale, we considered a probable reaction when a clinical event had a temporal relationship with drug administration, presenting or not an abnormal laboratory test, and which was unlikely to be attributed to other diseases or chemicals and showed clinical response on drug withdrawal.¹³

The study included patients over 18 years old of any sex, with a probable history of DHRs, who accepted to participate and signed an informed consent form (ICF). Exclusion criteria considered patients with unlikely or possible history of DHRs, patients with other chronic diseases such as psychosomatic diseases and routine use of analgesic drugs capable of interfering with QoL, and those who refused participation.

Translation of the DrHy-Q

Linguistic adaptation was based on a method suggested by the author of the original questionnaire, so as to obtain a translated version that is conceptually equivalent to the original questionnaire.

Translation from the original Italian version to Portuguese (Brazilian culture) was overseen by a team (two bilingual Italian translators and two allergist and immunologist physicians, who held technical knowledge in the area), in an independent manner and being informed of the research objectives.

We emphasized the need for the translation to be conceptual and not only literary, as well as for the

language to be colloquial and easily understandable. The translation encompassed the same concepts and items as the original instrument, with culturally relevant and accepted expressions.

Back translation of the DrHy-Q

The consensus version was back translated into Italian by another native Italian, bilingual Portuguese translator and was compared to the original version by a native Italian evaluator in order to revise the reference meaning of each item, in addition to detecting differences or discrepancies between both editions.

This back-translated version was also evaluated by the study team and by the author of the original questionnaire, thus proceeding to the cross-cultural adaptation phase.

Adaptation of the DrHy-Q

The consensus version was applied to a sample of 10 patients with the aim of evaluating whether the translation was easily understood, with simple and suitable language.

The patients were randomly selected in routine consultations. They were inquired as to difficulties in understanding the questions and answer options and when pertinent, their opinion was requested on which was the best way to make these questions.

The final version of the adapted DrHy-Q (DrHy-Qb) (Annex 1) comprised 15 items, with a total score that varied from 15 points (better QoL) to 75 points (worse QoL), employing a 5-point Likert scale.

Validation of the adapted DrHy-Qb

A convenience sample comprising 84 patients with probable history of DHRs answered the DrHy-Qb, along with the Brazilian version of a generic health instrument (SF-36).¹²

Psychometric measures taken during this validation process include construct validity, reliability, and reproducibility of the evaluated instrument.

Statistical analysis

The collected data were stored in a Microsoft Excel file and statistical analysis was performed with SPSS 25.0 software, at a significance level of 5% ($p < 0.05$).

Quantitative and qualitative variables were assessed in a descriptive manner. In addition, analyses of normality of the sample and homogeneity of variances were performed as a prerequisite for possible analyses of parametric and non-parametric correlations.

For construct evaluation of the questionnaire, we chose to calculate Pearson's correlation coefficient (r), which can vary from -1 to +1; values closer to 1 represent stronger correlations. Negative values correspond to inverse correlations.¹⁴

Cronbach's alpha reliability coefficient was used for assessing internal consistency. Results closer to 1 represent the most consistent variables.¹⁵ Values over 0.75 are considered acceptable.^{16,17}

For assessing the reproducibility of DrHy-Qb, we used the intraclass correlation coefficient (ICC), looking for consistency between when the questionnaire was first answered and when it was reapplied, after a period of 7 to 30 days. In order to achieve statistical validity, at least 20% of the sample is expected to be reevaluated. Results close to 1 indicate a strong correlation, that is, with less variability between the compared measures.^{18,19}

Ethical aspects

This research proposal was analyzed and approved by the Research Ethics Committee at UNIFESP and is registered at Plataforma Brasil (CAAE: 42295414.7.00005505). The integrity, privacy, and secrecy of patient information were rigorously respected. All research procedures were in agreement with resolution No. 466/12 and resolution No. 510/16 of the National Health Council on research with human beings. All participants signed the ICF.

Results

Assessing conceptual, item, and semantic equivalence of the DrHy-Qb

Out of ten selected patients, seven demonstrated they understood all items of the DrHy-Qb at the cultural adaptation phase. Three patients suggested semantic changes to questions, considering more appropriate terms and words for a better understanding and approximation to the Brazilian reality.

During the validation phase, some interviewees had difficulty distinguishing the inquired feelings:

anxiety, anguish, fright, worry, and fear. The mean application time was 7.5 minutes for patients who understood and filled out the questionnaire, whereas the mean time for those who required help by an interviewer for reading the questions was 24 minutes. All patients answered the DrHy-Qb questions.

Socioeconomic and clinical characteristics of the sample

Women comprised 69% ($n = 58$) of the sample, and the mean age of the population was 40.3 years (median [SD], minimum-maximum; 36 years [15.19], 18-72 years). We decided to read the questions for 21.4% of the patients ($n = 18$) who were not able to read or write and had basic knowledge on the disease. The other socioeconomic characteristics are described in Table 1.

The most frequent clinical manifestation was isolated palpebral angioedema, which occurred in 40.5% of the cases ($n = 34$), followed by anaphylaxis in 35.7% ($n = 30$). Hypersensitivity reactions were related with the number of drugs, number of episodes, and class of the implicated drugs (Table 2).

Reliability assessment of the DrHy-Qb

By calculating Cronbach's alpha coefficient, we demonstrated that the internal consistency between the 15 items of the DrHy-Qb was excellent ($\alpha = 0.936$).

Similarly, the DrHy-Qb was shown to be precise when confirming item reliability, that is, all questions contributed for a good accuracy when comprising the questionnaire (Table 3).

Assessing reproducibility via the intraclass correlation coefficient (ICC)

Twenty-three patients (27.3%) were randomly selected and answered the DrHy-Qb again after a period that varied from 7 to 30 days after first assessment. In this study, the agreement of questionnaire reproducibility was classified as excellent.

The ICC showed a correlation of $r = 0.984$ (95%CI = 0.963-0.993, $p < 0.001$), confirming an excellent agreement for DrHy-Qb reproducibility.

Table 1

Demographic and socioeconomic characteristics of the studied population

Characteristics	N (%)
Patients	84 (100)
Sex	
Female	58 (69)
Male	26 (31)
Age group	
18-29 years old	26 (31)
30-59 years old	44 (52.4)
60 years old or older	14 (16.7)
Education	
Illiterate	18 (21.4)
Lower secondary education	10 (11.9)
Upper secondary education	21 (25)
Undergraduate/graduate education	35 (41.7)
Instrument application	
Self-administered	66 (78.6)
Required help reading	18 (21.4)

Construct evaluation of DrHy-Qb

The mean DrHy-Qb score was 43.19 points (median [SD], minimum-maximum; 44 [13.95], 15-70) and the mean SF-36 total score was 108.44 points (median [SD], minimum-maximum; 111.55 [18.48], 60.5-139) (Table 4). It is important to note that higher DrHy-Qb scores, as well as lower SF-36 scores, represent worse QoL.

Pearson's correlation coefficient showed a moderate negative correlation (the instruments are inversely proportional) between mean DrHy-Qb scores and mean SF-36 total scores ($r = -0.394$; $p < 0.01$). Considering the domains, no significance was observed in "physical role limitations" ($p = 0.11$) and "vitality" ($p = 0.07$). The other domains, on the other hand, showed a significant moderate and negative correlation with mean DrHy-Qb scores (Table 5).

Table 2
Clinical characteristics of the studied population

Characteristics	N (%)
Number of episodes	
1 episode	31 (36.9)
2 or more episodes	53 (63.1)
Number of drugs	
1 drug	30 (35.7)
2 or more drugs	54 (64.3)
Type of drugs	
NSAIDs	58 (69.0)
Beta-lactams	5 (6.0)
Local anesthetics	5 (6.0)
Perioperative anaphylaxis ^a	13 (15.5)
Other ^b	3 (3.6)
Type of reaction	
Anaphylaxis	30 (35.7)
Angioedema of the lips	19 (22.6)
Angioedema of the larynx	10 (11.9)
Angioedema of the tongue	1 (1.2)
Palpebral angioedema	34 (40.5)
Severe delayed adverse cutaneous reactions	0 (0.0)
Respiratory symptoms	1 (1.2)
Maculopapular exanthem	8 (9.5)
Urticaria	20 (23.8)

NSAIDs = non-steroidal anti-inflammatory drugs.

^a Drugs involved in perioperative anaphylaxis: benzodiazepines, neuromuscular blocking agents, hypnotics, opioids, cholinesterase inhibitors, antiemetics, and antiseptics.

^b Others: proton pump inhibitors and radiocontrast agents.

Discussion

Translation and cross-cultural adaptation

In the translation phase, the back-translated version was compared to the original source version in order to detect misunderstandings, translation errors, or imprecisions of the new questionnaire. When developing an instrument by consensus building, translation performed by a committee approach

reduces the cultural and social bias that can happen when only one or two translators are responsible for the translation.

Even though all versions of the DrHy-Q were translated from the original Italian questionnaire, each adaptation into different cultures could have altered the questionnaire content and consequently its objective assessment. This is minimized when consulting the author of the original questionnaire. After validating the Brazilian version, we named it DrHy-Qb for better differentiating it from other available cultural versions.

Some interviewees had difficulty distinguishing feelings inquired about in the DrHy-Qb: anxiety, anguish, fright, worry, and fear. Even though all these terms are related to negative feelings, they are not considered synonyms.^{20,21}

According to the Brazilian Institute of Geography and Statistics, 11.3 million people over 15 years old could not read nor write in 2018.²² It is thus expected that some patients would face limitations in understanding the questionnaire. This way, we considered helping 18 patients read the instrument for a better understanding of the process; a mean duration of 24 minutes was required for answering the questionnaire. The other patients, with education levels ranging from lower secondary education to graduate education, had no difficulties answering the questionnaire.

The Thai version reported a mean time of approximately 7.4 minutes, which was similar to that observed in our study.¹⁰ The other DrHy-Q validation studies did not specify the duration of questionnaire application.⁶⁻¹¹

Regarding data collection, the Thai study reported low rates of unanswered items: around 1.2% of the sample.¹⁰ The Dutch study showed non-responses in 42% of the cases; this was expected since data collection was performed via e-mail.⁸ In our study, all patients fully answered the Brazilian version (DrHy-Qb).

Assessment of the questionnaire's psychometric properties

The psychometric assessment data obtained in our study showed that DrHy-Qb is in line with the results of other validation studies of this instrument.⁶⁻¹¹

For construct evaluation, we used Pearson's correlation coefficient (*r*) because this analysis has

Table 3

Cronbach's alpha reliability coefficient for each item of the DrHy-Qb

Item	Cronbach's alpha if the item was excluded
1. Since I am unable to take drugs every illness limits me more than other people.	0.933
2. I am afraid of being administered a drug during an emergency to which I am allergic.	0.932
3. I feel frightened due to my problem of allergy reaction.	0.928
4. The problem of adverse reaction to drugs affects my life.	0.930
5. I would like the allergist's opinion before taking drugs prescribed by other specialists.	0.935
6. Even a little discomfort for me is a problem.	0.931
7. The fact that I cannot use medication safely made me feel different from others.	0.935
8. I feel anxious due to my problem of allergy reaction.	0.928
9. For each disease I would be confident that there is a drug that I can safely take.	0.933
10. I am afraid I could not deal with the pain.	0.931
11. I feel anguished due to my problem of allergy reaction.	0.928
12. I worry every time I take a drug different from ones that cause my allergic reactions.	0.930
13. I give up leisure activities (sport, vacations, trips) because of my problem.	0.936
14. I'm in a bad mood due to my problem of allergy reaction.	0.933
15. The idea of taking a medicine makes me feel anxious.	0.933

DrHy-Qb – Drug Hypersensitivity Quality of Life Questionnaire (Brazilian version).

two dependent quantitative variables (DrHy-Qb and SF-36). We observed a negative correlation, that is, an inverse proportionality, which is in agreement with the interpretation that higher DrHy-Qb scores represent worse patient QoL, and the higher the absolute values of SF-36 total scores and scores by domain, the better the patient's QoL.

No patient reported regular use of drugs for treating any physical disease. In this study, data showed a lack of correlation between possible physical role limitations and vitality and a compromised patient QoL.

Construct evaluation via Pearson's correlation coefficient between DrHy-Qb scores and mean SF-36 total scores was shown to be moderate and negative ($r = -0.394$; $p < 0.01$), and showed that specific aspects

Table 4

Quality of life scores according to each applied questionnaire

Questionnaire (n=84)	Score		
	Mean	Median	(min - max, SD)
DrHy-Qb	43.19	44.00	(15 - 70; 13.95)
SF-36	108.44	111.55	(60.5 - 139; 18.48)

DrHy-Qb: DrHy-Qb – Drug Hypersensitivity Quality of Life Questionnaire (Brazilian version); SF-36: The Medical Outcomes Short-Form Health Survey (Brazilian version).

Table 5

Pearson's correlation coefficient between DrHy-Qb and SF-36 (total score and by domain)

	DrHy-Qb	
	Pearson's correlation (r)	p
SF-36 total score	-0.394	< 0.01
SF-36 domains		
Physical functioning	-0.314	< 0.01 ^a
Physical role limitations	-0.180	0.10
Pain	-0.295	< 0.01 ^a
General health	-0.238	0.03 ^a
Vitality	-0.207	0.05
Social functioning	-0.393	< 0.01 ^a
Emotional role limitations	-0.218	0.04 ^a
Mental health	-0.400	< 0.01 ^a

DrHy-Qb – Drug Hypersensitivity Quality of Life Questionnaire (Brazilian version); SF-36: Medical Outcomes Short-Form Health Survey (Brazilian version).

^a Significant correlations ($p < 0.05$).

of the HRQoL of patients with DHR episodes were not adequately detected by the generic instrument (SF-36). This result corroborates data found by other DrHy-Q validation studies.⁶⁻¹¹

The reliability assessment of DrHy-Qb by Cronbach's alpha coefficient showed how consistent this instrument is. If the answers were to be discrepant, the instrument could be unreliable for measuring what it intends to. Some questions are capable of increasing questionnaire consistency, while others are able to decrease it. Our results indicated a global α of 0.936, showing excellent consistency between the 15 questionnaire items.

By comparing the global alpha to the alpha obtained when excluding each question, we were able to determine whether removing an item would increase overall questionnaire reliability in case the resulting alpha was higher than the global coefficient. In our study, all questions presented a similar and important behavior when comprising the DrHy-Qb,

with an α per item above 0.928 and a global α of 0.936. DrHy-Q also presented high reliability in other studies.⁶⁻¹¹

Statistical analyses showed that DrHy-Qb met reproducibility standards for validating HRQoL instruments, which was similar to previous studies.⁶⁻¹¹ In order to avoid sample losses, some patients answered the questionnaire again after a period of 7 to 30 days.

All of them were inquired on the occurrence of any significant life and health changes in the previous week, similarly to what was performed in the Thai study,¹⁰ and time did not determine the impact on patients' HRQoL.

Our test-retest assessment, applied in 27.3% of the sample, had an excellent result that was confirmed by calculating the ICC ($r = 0.984$; 95%CI = 0.963-0.993, $p = 0.0001$). The other DrHy-Q validations also reapplied the questionnaire in around the same sample percentage and obtained similar results.⁶⁻¹¹

Scores obtained in the DrHy-Qb

Comparing DrHy-Qb scores with those obtained in studies of other countries helped us investigate cultural differences and impacts on HRQoL. However, the Dutch validation study converted the scale for the DrHy-Q score to a 0-100 format, where the maximum score indicated a compromised QoL.⁸

Using the tool as it was described and developed is important for a better comparison of case series and their respective results. For this reason, the total DrHy-Q score was converted into a 0-100 scale, where 100 points indicate a more severely compromised HRQoL, and to the original 15-75 scale, where 75 points indicate a worse QoL.⁶ Therefore, we were able to analyze median DrHy-Q scores in different populations where this tool was validated (Table 6).

We used the following formula for converting the DrHy-Q score into a 0-100-point scale:

$$\text{DrHy-Q} = \frac{(\sum \text{items} - 15) * 100}{[75 - 15]}$$

The median DrHy-Qb score was 48 points. For reformulating the DrHy-Q score in a 15-75-point scale, we used the formula:

$$\text{DrHy-Q} = [\sum \text{items} * \left(\frac{75 - 15}{100 - 0} \right)] + 15.$$

The median DrHy-Qb score was 44 points (Table 6).

The median DrHy-Q score was not described in the Thai study, only the means for the studied variables.¹⁰ In the Dutch study, on the other hand, lower values were justified by the fact that this was a retrospective study where diagnoses and counseling were performed before questionnaire application, as opposed to what happened with the Italian and Turkish versions. Moreover, the Dutch people displayed more healthy perceptions when compared to the other studied countries, such as a lower use of medicines. These cultural and social differences may influence DrHy-Q results.⁸

Table 6

Comparison of median DrHy-Q scores in different countries

Reference	Country	Median DrHy-Q score (scale)	
		(15-75)	(0-100)
Baiardini and colleagues, 2011 ⁶	Italy	37	36
Bavbek and colleagues, 2016 ⁷	Turkey	35	33
Gastaminza and colleagues, 2016 ⁹	Spain	29	23
Moayeri and colleagues, 2017 ⁸	The Netherlands	22	12
DrHy-Qb (this study)	Brazil	44	48
Dias de Castro and colleagues, 2021 ¹¹	Portugal	37	36

The median DrHy-Qb score, when compared to results obtained in other countries, was considered high, probably due to easy access to medicines (many of them can be purchased without medical prescription) and consequent self-medication, increasing the frequency of exposure to drugs. Another associated factor could also be the increase in indiscriminate use of medicines in Brazil.²³

As opposed to other retrospective studies where the patient is approached years after the reactions or medical counseling, one of the advantages of our study was the fact that patients were assessed while the diagnostic hypothesis was raised and before performing skin or provocation tests. This way, the risk of influencing the questionnaire results is reduced.

All patients adequately answered all items of the DrHy-Qb instrument and no losses were observed in our sample. Another aspect that did not contribute to possible biases was the fact that we did not approach psychosomatic or psychiatric diseases, nor conditions related to chronic pain and frequent use of painkillers. These confounding factors could compromise this specific HRQoL assessment.

Limitations of the study and applicability of the validated instrument

One of the limitations of our study was the fact that it was not performed alongside other drug allergy referral centers in Brazil, which would provide a larger case series and a broader representation of patients with DHRs.

Ethnic and socioeconomic differences, as well as participation of patients with severe delayed cutaneous reactions, were not assessed in this study. These topics will be investigated in the future.

Conclusions

This study enabled adequate translation and cultural adaptation of the DrHy-Q questionnaire, along with assessment of its psychometric properties according to guidance by the authors who developed it.⁶ The Portuguese version of the DrHy-Q (Brazilian culture, DrHy-Qb) presented excellent reliability, construct validity, and reproducibility, which enables its use for evaluating the QoL of patients with drug hypersensitivity, in addition to directing treatment or psychological support to those who most need it. DrHy-Qb is a useful tool for assessing HRQoL in future Brazilian studies.

Nome: _____ Data: ____ / ____ / ____

As reações adversas aos medicamentos podem influenciar o bem estar físico e psíquico das pessoas. Pedimos que você indique abaixo as dificuldades que encontrou devido a esse problema.

	Nenhuma	Pouca	Razoável	Muita	Muitíssima
1. Como não posso tomar medicamentos, qualquer doença é mais limitante para mim que para outras pessoas.	1 ()	2 ()	3 ()	4 ()	5 ()
2. Tenho medo que numa emergência me receitem um medicamento ao qual sou alérgico(a).	1 ()	2 ()	3 ()	4 ()	5 ()
3. Fico apavorado(a) por causa do meu problema com os medicamentos.	1 ()	2 ()	3 ()	4 ()	5 ()
4. O problema das reações aos medicamentos condiciona minha vida.	1 ()	2 ()	3 ()	4 ()	5 ()
5. Gostaria da opinião do alergista antes de tomar medicamentos receitados por outro especialista.	1 ()	2 ()	3 ()	4 ()	5 ()
6. Até um pequeno mal estar se torna um problema para mim.	1 ()	2 ()	3 ()	4 ()	5 ()
7. O fato de não poder tomar tranquilamente os medicamentos me faz sentir diferente dos outros.	1 ()	2 ()	3 ()	4 ()	5 ()
8. Fico ansioso(a) por causa do meu problema de reações aos medicamentos.	1 ()	2 ()	3 ()	4 ()	5 ()
9. Para qualquer doença gostaria de ter certeza de que existe um medicamento que posso tomar tranquilamente.	1 ()	2 ()	3 ()	4 ()	5 ()
10. Tenho medo de não poder tratar a dor.	1 ()	2 ()	3 ()	4 ()	5 ()
11. Fico angustiado(a) por causa do meu problema de reações aos medicamentos.	1 ()	2 ()	3 ()	4 ()	5 ()
12. Fico preocupado(a) toda vez que tenho que tomar um medicamento, mesmo diferente daquele que causou a reação alérgica.	1 ()	2 ()	3 ()	4 ()	5 ()
13. Renuncio a ocasiões de lazer (esportes, férias, viagens) devido o meu problema.	1 ()	2 ()	3 ()	4 ()	5 ()
14. Me sinto deprimido(a) por causa do meu problema de reações aos medicamentos.	1 ()	2 ()	3 ()	4 ()	5 ()
15. A ideia de uma injeção ou aplicação de medicamento na veia me deixa ansioso.	1 ()	2 ()	3 ()	4 ()	5 ()

Annex 1

Portuguese version of the Drug Hypersensitivity Quality of Life Questionnaire (Brazilian culture) - DrHy-Qb

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Detection of the soy allergen Gly m 1 in the air in Maringá-PR

Presença do alérgeno de soja Gly m 1 no ar em Maringá-PR

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ABSTRACT

Introduction: Knowledge of local aerobiology is essential for allergists. Because airborne allergens can sensitize the population and lead to allergic respiratory diseases, they must be routinely monitored for the effects of climate change, pollution, and agroindustry. **Objective:** To verify the airborne presence and concentration of the main soy hull dust allergen (Gly m 1) in Maringá, PR, Brazil and possible associations with climatic factors. Soybeans were selected due to the high prevalence of this crop in this region. To date, only 1 pilot study (conducted by our group) has evaluated this allergen's presence in Brazil. **Methods:** Atmospheric material was collected between March 2017 and March 2018 in 24- or 48-hour intervals, totaling 70 samples, of which 10 were excluded due to technical problems. The samples were tested for Gly m 1 using enzyme-linked immunosorbent assay, and all samples showed detectable levels of the allergen. **Results:** The median concentration of Gly m 1 was 4.89 ng/m³, with values ranging from 0.66 ng/m³ to 1826.1 ng/m³. Of the 60 samples, 23% showed values > 90 ng/m³, with June 2017 and March 2018 having the highest concentrations. There was a positive correlation between Gly m 1 concentration and maximum, mean, and minimum temperatures, relative humidity, wind, and insolation. **Conclusion:** The data show that the population is constantly exposed to the Gly m 1 allergen, sometimes at high levels, which may lead to sensitization and symptoms.

Keywords: Allergens, soy, air samples, ELISA.

RESUMO

Introdução: O conhecimento da aerobiologia local é fundamental para o alergista. Os aeroalérgenos são capazes de sensibilizar e levar ao desenvolvimento de doenças respiratórias alérgicas, portanto devem ser monitorados rotineiramente, tendo em vista possíveis mudanças locais conforme alterações climáticas, poluição e atividades agroindustriais. **Objetivo:** Verificar a presença e concentração do alérgeno principal da poeira da casca da soja (Gly m 1) na atmosfera da cidade de Maringá-PR e possíveis associações aos fatores climáticos. A escolha da soja deve-se a alta prevalência desta cultura no Brasil e nesta região do país. Até o presente momento, há apenas um estudo piloto feito por este mesmo grupo avaliando a presença deste alérgeno no Brasil. **Métodos:** Foram realizadas coletas de material atmosférico, durante o período de março de 2017 a março de 2018, durante 24 ou 48 horas distribuídas no decorrer do período, totalizando 70 amostras, das quais 10 foram excluídas por problemas técnicos de coleta. As amostras foram avaliadas pelo método ELISA (*Enzyme linked immunosorbent assay*) para Gly m 1, sendo que todas as amostras apresentaram níveis detectáveis do alérgeno. **Resultados:** A mediana de concentração de Gly m 1 foi de 4,89 ng/m³. Os valores encontrados variaram de 0,66 ng/m³ a 1826,1 ng/m³. Das 60 amostras analisadas, 23% delas apresentaram valores superiores a 90 ng/m³, sendo os meses de junho/2017 e março/2018 com concentrações mais elevadas. Houve correlação positiva das concentrações de Gly m 1 com as temperaturas máxima, média e mínima, umidade relativa, vento e insolação. **Conclusão:** Os dados evidenciam exposições constantes da população ao alérgeno do Gly m 1, por vezes em níveis elevados possivelmente capazes de gerar sensibilização e sintomas.

Descritores: Alérgenos, soja, amostras de ar, ELISA.

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Introduction

Changes in the distribution of aeroallergens occur gradually over time and require monitoring in order to identify individuals' environmental exposure, as well as possible new sensitization and triggers of respiratory symptoms. The emergence of molecular allergology has allowed us to identify, with sensitivity and specificity, the sensitization profile of individuals. Likewise, it has broadened our understanding of allergens in each location, their concentrations and potential population exposures.^{1,2}

In this context, the main soy hull dust allergen, Gly m 1, began to be investigated, particularly in European countries, because of its potential relationship with asthma outbreaks that had occurred at the seaports of some cities related to the unloading of soybeans into silos.³⁻⁷ With advances in research in the field, allergen dispersion was found to be associated with local climate issues, as well as with population genetic aspects.⁸⁻¹⁰ New strategies to avoid the spread of this allergen began to be used in European ports, such as the installation of bag filters in the silos, which led to a significant reduction of Gly m 1 in the atmosphere and prevented new asthma outbreaks.¹¹

However, despite the importance of Gly m 1 as an aeroallergen, research has not advanced to investigate the presence of this allergen in areas of potential dispersion. In the last decade, no aerobiological survey of this allergen was conducted in any location in the world, despite the fact that Gly m 1 is a sensitizer both in occupational exposure and indirect exposure.^{6,7,10,12} In Brazil, Pinto et al.¹² showed frequent sensitization to soybean in workers and residents of soy-producing areas, but they did not assess allergen concentrations in the atmosphere. In a pilot survey of Gly m 1 conducted in 2016 by our research group, we demonstrated the presence of this allergen in the atmosphere, which motivated the current study.¹³

Methods

Air samples were collected periodically from March 2017 to March 2018 in pure borosilicate glass fiber filters (Pallflex®), 47 mm in diameter, with heat-resistant, moisture-resistant fluorocarbon coating (tetrafluoroethylene, TFE). During air sampling, moisture variations in the air or gases did not cause chemical reactions in the filter.

A total suspended particulate sampler capable of capturing particles of different sizes was used. The

sampler was placed in the urban area of Maringá, a city in the state of Paraná, south of Brazil, at 23.41° south latitude, 51.97° west longitude, and 537 m altitude in an external environment at 90 cm above the ground. Seventy samples were collected at 24- or 48-hour intervals. The duration of each collection was based on our pilot study and on previous studies that showed the presence of Gly m 1 in 24-hour samples.^{7,9,10,13} Samples that experienced technical problems, such as power outages, excessive rainfall, and inadequate collection time, were excluded. The collection point was located at a distance of 2.5 km, in a straight line, from the major soybean processing cooperative in the region. The collected filters were stored at -20 °C until analysis.

To prepare the samples, the filters were cut into 3 cm² pieces and eluted in a solution of NH₄HCO₃ with 1% bovine serum albumin (BSA) and 0.1% Tween 20® (Polysorbate), homogenized in an orbital shaker at 4 °C overnight, for a final volume of 3 mL. After the elution process, the material was centrifuged twice, and the supernatant used for analysis was extracted by enzyme-linked immunosorbent assay (ELISA).

The ELISA was performed with plates presensitized with anti-Gly m 1 monoclonal antibody (ALK-Abello®), as described by Gonzalez et al.⁶ This method is highly specific with a rare possibility of cross-reactivity with other allergens. Plates were previously washed, and samples, standard curve, controls and blank filters were added in duplicate. Subsequently, the biotinylated detection antibody (ALK-Abello®) conjugated to streptavidin-peroxidase (STAV-PO, Thermo Fisher-Scientific®) was added to the plates. Finally, 3,3',5,5'-tetramethylbenzidine (TMB) was added to the plates incubated in a dark environment, and the reaction was interrupted with 4NH₂SO₄. The reading was performed by spectrophotometry at 490 to 650 nm wavelengths, inferring the concentrations.

The following meteorological data were collected daily from March 2017 to March 2018 from the National Institute of Meteorology (INMET) website at the Maringá weather station (WMO: 83767; latitude: -23.4; longitude: -51.91; altitude: 542 m): maximum, minimum, and average compensated temperature; relative humidity (RH); precipitation; wind speed and direction; and insolation.

Results

A total of 70 filters were collected during the 12-month study period (from March 29, 2017, to March

26, 2018). The collections were standardized to occur in 1 or 2 days, with samples collected at an average of 24 hours and 6 minutes or 48 hours and 20 minutes, respectively. The volume of air sampled in each filter was, on average, 1.13 m³ of air per hour. Filters were collected every month, with a mean and median interval between collections of 5 days. Of the total of 70 filters, 10 were excluded from the analysis due to inappropriate collection.

ELISA concentrations of Gly m 1 (ng/mL) were converted to ng of allergen per m³ of air, as this is the most commonly used unit of measurement. The median concentration of Gly m 1 was 4.89 ng/m³. All samples showed the presence of Gly m 1, with concentrations ranging from 0.67 ng/m³ to 1826.1 ng/m³ (Figure 1).

The 24- and 48-hour filters showed similar concentrations ($p=0.32$; Mann-Whitney test), with a median concentration of 4.4 ng/m³ in the 24-hour filters and of 6.3 ng/m³ in the 48-hour filters. The analysis of the two groups, excluding outliers, showed a median concentration of 4.03 ng/m³ in the 24-hour group and 3.09 ng/m³ in the 48-hour group, once again demonstrating homogeneity between the groups ($p=0.157$; Mann-Whitney test). The first quartile value of 2.24 and the third quartile value of 91.4 were used to calculate outliers. Outliers were all values above 225.2 ng/m³, totaling 52 samples.

Regarding the meteorological data on collection days, the average temperature was 22.4 °C, RH was 68.5%, and insolation was 7.7 hours. The data were similar to those of other days of the year when no collection was performed. The same was true for wind speed, with an average of 1.48 m/s on collection days, which is considered light air. As for wind direction, there was a predominance of east and northeast winds on the collection days. It should be noted that the cooperative is located between north and northeast to the collection point.

Gly m 1 concentrations were positively correlated with temperature and RH (Table 1). The same statistical analyses performed excluding outliers showed a positive correlation of Gly m 1 concentrations with temperature, RH, insolation, and wind speed (Table 2).

Discussion

The soybean dust allergen can cause respiratory symptoms as confirmed by bronchial provocation tests.^{14,15} However, the impact of the presence of Gly m 1 in the atmosphere remains unknown. Based on data from outbreaks in Barcelona in the 1990s, soybean dust allergenicity is believed to occur in 1 in every 1700 people.¹⁶ Climate factors and particular characteristics of the population are known to

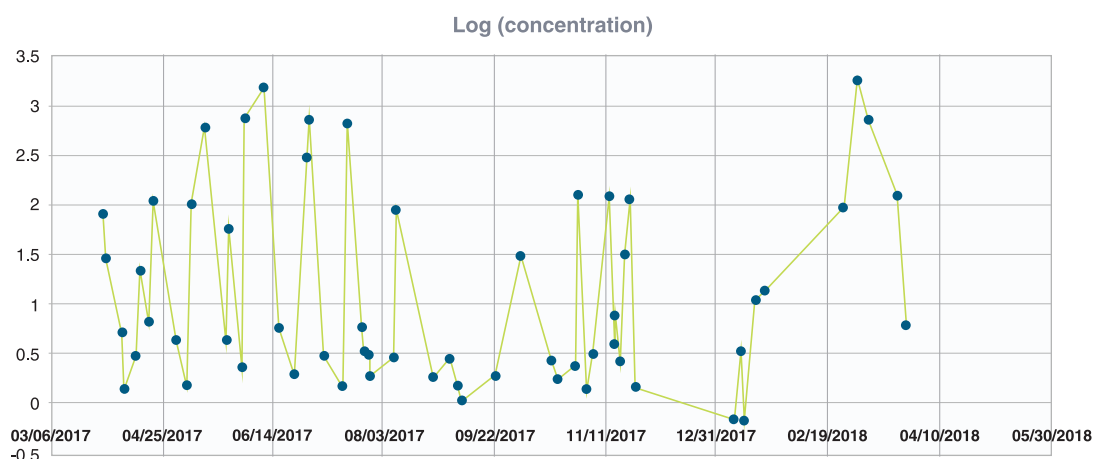


Figure 1

Gly m 1 concentrations on a logarithmic scale (Log¹⁰) in the 60 samples

Table 1Coefficients of correlation (R) and determination (R²) between Gly m 1 concentrations and meteorological data

	Gly m 1 concentration (Log ¹⁰)			Gly m 1 concentration (Cube root)		
	R	R ²	p	R	R ²	p
Maximum temperature	0.73	0.53	< 0.001	0.72	0.51	< 0.001
Minimum temperature	0.71	0.5	< 0.001	0.7	0.49	< 0.001
Average temperature	0.72	0.51	< 0.001	0.71	0.5	< 0.001
Relative humidity	0.75	0.56	< 0.001	0.75	0.56	< 0.001
Precipitation	0.37	0.14	0.003	0.4	0.16	0.001
Insolation	0.66	0.43	< 0.001	0.64	0.41	< 0.001
Wind speed	0.67	0.45	< 0.001	0.69	0.48	< 0.001
Wind direction	0.32	0.10	0.012	0.32	0.10	0.012
Multivariate ^a	0.79	0.63	NS	0.79	0.62	NS

^a With all climate variables.

NS = non-significant.

Table 2Coefficients of correlation (R) and determination (R²) between Gly m 1 concentrations (without outliers) and meteorological data

	Gly m 1 concentration (Log ¹⁰)			Gly m 1 concentration (Cube root)		
	R	R ²	p	R	R ²	p
Maximum temperature	0.77	0.59	< 0.001	0.86	0.73	< 0.001
Minimum temperature	0.76	0.57	< 0.001	0.85	0.72	< 0.001
Average temperature	0.76	0.58	< 0.001	0.85	0.73	< 0.001
Relative humidity	0.74	0.55	< 0.001	0.83	0.69	< 0.001
Precipitation	0.29	0.08	0.038	0.36	0.13	0.008
Insolation	0.71	0.51	< 0.001	0.79	0.62	< 0.001
Wind speed	0.65	0.42	< 0.001	0.74	0.55	< 0.001
Wind direction	0.22	0.05	0.11	0.28	0.08	0.045
Multivariate ^a	0.82	0.67	0.008 for WS	0.89	0.78	0.010 for WS

^a With all climate variables.

WS = wind speed.

interfere with this sensitization and certainly with symptom presentation.^{10,17,18} Furthermore, soybean hull allergenicity can be affected by heat during grain storage and transport, which can produce new allergen determinants or increase epitope exposure by conformational changes.¹⁹

Some studies have evaluated ELISA-determined sensitization to Gly m 1 in port populations and produced important results correlating the allergen with asthma outbreaks.^{3,20,21} Codina et al.²² and Pendino et al.²³ evaluated sensitization to soybean hull extract by skin prick test in adults and children, respectively, with asthma. Both studies showed sensitization to soybean hulls even in populations without direct exposure to soybeans. However, all those tested were polysensitized, also showing reactivity to mites, fungi, or pollens.

In Brazil, Pinto et al.¹² identified that symptomatic individuals who lived in rural areas within soybean belts showed 28% of sensitization to soybean by skin tests with total extract, and 5% of them were monosensitized to soybean. Likewise, among residents of the manufacturing area and truck drivers transporting soybeans, sensitization was high (22% for both groups), but not exclusive, as they were also sensitized to storage mites and fungi that colonize the soybeans. Therefore, there is a group of polysensitized atopic individuals in whom soybean can be an additional sensitizer in a context in which mites and fungi play the main role as protagonists of allergic respiratory manifestations.

The Gly m 1 levels detected in the atmosphere are higher near soybean handling sites. Measurements were 12 times higher in a port area than 5 km away from the port.¹⁰ In the current study, the collection point was at an intermediate distance from the cooperative (2.5 km). Despite a relative distance, we showed detectable Gly m 1 levels in all samples collected for 12 months. Soybean hull dust can also be produced during the soybean harvest period and at all stages of soybean processing,^{9,10,24} which explains the continuing and not only seasonal presence of Gly m 1 in the atmosphere of Maringá. Although the harvest period occurs between January and March, the cooperative processes soybeans throughout the year.

In the current study, we identified some positive correlations when the concentrations were transformed for data analysis (Log¹⁰ and cube root). We decided to perform these data transformations because of the wide dispersion of concentration values (ranging

from 0.67 ng/m³ to 1826.1 ng/m³). Correlation and regression analyses are influenced by the presence of extreme values, and data transformation can reduce the bias effect. Correlations were stronger when outliers were removed. There was a positive correlation between Gly m 1 concentrations (in cube root) and temperature ($R \geq 85\%$), RH ($R = 83\%$), insolation ($R = 79\%$), and wind speed ($R = 74\%$). The univariate linear regression models using again the cube root-transformed concentrations showed $R^2 > 70\%$ for temperature and $R^2 = 69\%$ for RH. Similarly, in a study conducted in the port of Ancona, Italy, Antonicelli et al.¹⁰ observed in the multivariate linear regression model that the Gly m 1 concentrations were influenced by temperature and RH.

High atmospheric pressure, calm winds and wind direction have been potentially associated with greater dispersion of the Gly m 1 allergen.^{25,26} In the current study, there was a positive correlation between wind speed and Gly m 1 concentrations. However, during the study period, wind speed was considered calm, ranging from light air to light breeze. This may have been a contributing factor to the continuing spread of the allergen. Wind direction was not correlated with Gly m 1 concentrations, but there was a predominance of winds from the cooperative toward the collection point, which may have favored the daily presence of detectable Gly m 1 levels.

Although there is controversy about the Gly m 1 values capable of triggering symptoms, in the current study, some values were high. Of the 60 samples analyzed, 14 (23%) showed values greater than 90 ng/m³, with a peak of 1826 ng/m³ in March 2018. It should be noted that in almost all months at least one measurement was greater than 90 ng/m³, except for September 2017 and January 2018, which showed the lowest monthly concentrations, ranging from 1.09 to 2.85 ng/m³ and from 0.66 to 13.9 ng/m³, respectively. In contrast, the highest Gly m 1 levels were detected in June 2017 and March 2018. In June 2017, values ranged from 1.98 to 1549 ng/m³ (median 303.44 ng/m³), and in March 2018, values ranged from 6.35 to 1826 ng/m³ (median 428 ng/m³).

The low Gly m 1 levels found in January 2018 can be attributed to precipitation, which was intense this month, with an average of 21 mm on collection days, in line with previous studies.⁹ Higher values observed in March 2018 coincided with the soybean harvest period. Gijzen et al.⁹ also observed that peak Gly m 1 levels occurred during the harvest month (reaching 73 ng/m³). The presence of nearby crops and the

pattern of harvest operations may have influenced the Gly m 1 levels found.

Some limitations should be addressed in this study. Samples were collected at a single site in the city and reflect only the situation of that collection point. The sampler was placed at a height of only 90 cm above the ground, which might have negatively influenced the sampling due to nearby objects. Collection days were chosen by convenience. Very rainy days were automatically discarded due to the impossibility of exposing the device to heavy rainfalls. The 48-hour collections, in particular, may have had losses of protein content due to prolonged exposure to high average temperatures. However, the 24- and 48-hour groups showed similar concentrations. In addition, climate data were provided only at specific time points (2 or 3 times a day) and averaged.

The present Gly m 1 findings need to be further explored. The use of ELISA to assess Gly m 1 in individuals with documented sensitization to soybean (by skin test or specific IgE) could determine the actual sensitization in this population, whether it occurs through food, the airway, or both. Another point to be considered and investigated is whether individuals sensitized through the airway could have soybean-related food symptoms and vice versa. Performing nasal, conjunctival or bronchial provocation tests with the soybean allergen may elucidate the true impact of Gly m 1 dispersion in the atmosphere on soybean-sensitized individuals.²⁷

Despite the limitations reported, the study was innovative in performing environmental analyses of soybean hull dust allergens for the first time in Brazil. Studies of environmental aeroallergens are essential for increasing allergists' awareness, enabling them to better understand the environment to which their patients are exposed, identifying risks, promoting prevention, and allowing personalized treatment. In terms of public health, awareness of these exposures allows the allergists to collaborate with other professionals (biologists, environmentalists, and environmental engineers), as it strengthens and encourages discussions in the political sphere about climate change, pollution, gas emissions, and all types of aerobiological monitoring.

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Polymorphism in cytolytic pathway genes in patients with pediatric inflammatory multisystemic syndrome temporally associated with SARS-CoV-2

Polimorfismos em genes da via citolítica em pacientes com síndrome inflamatória multissistêmica pediátrica temporariamente associada ao SARS-CoV-2

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ABSTRACT

Introduction: Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) is a systemic hyperinflammatory disease that occurs in a small number of children after being infected with SARS-CoV-2. Macrophage activation syndrome, an aggressive condition characterized by the excessive inflammation and activation of well-differentiated macrophages, has been shown to occur in patients infected by SARS-CoV-2. Considering the clinical and pathophysiological similarities between these diseases, our main objective was to determine whether gene polymorphisms associated with macrophage activation syndrome were also present in patients with PIMS-TS. **Methods:** DNA from 10 pediatric patients with PIMS-TS (case group) and ten COVID-19 patients without PIMS-TS (control group) were genotyped by Real-time PCR analysis (TaqMan[®]) for single nucleotide polymorphisms (SNP) in four genes associated with macrophage activation syndrome: *perforin 1 (PRF1)*, *granzyme B (GZMB)*, *syntaxin 11 (STX11)*, and *syntaxin binding protein 2 (STXBP2)*. The SNP analysis was performed using the additive, dominant, and recessive models. **Results:** A significantly higher frequency of an SNP (C wild allele in rs6573910) in the *GZMB* gene was observed in both the additive and dominant models in the PIMS-TS group than controls. A borderline significant difference was also observed for the G allele in rs7764017 of the *STX11* gene in the PIMS-TS

RESUMO

Introdução: A síndrome multissistêmica inflamatória pediátrica temporariamente associada ao SARS-CoV-2 (SIMP-TS) é uma doença hiperinflamatória sistêmica que ocorre em um pequeno número de crianças após serem infectadas pelo SARS-CoV-2. A síndrome de ativação de macrófagos (SAM), uma condição agressiva caracterizada pela inflamação excessiva e ativação de macrófagos bem diferenciados, demonstrou ocorrer em pacientes infectados por SARS-CoV-2. Considerando as semelhanças clínicas e fisiopatológicas entre essas doenças, neste estudo o nosso principal objetivo foi determinar se polimorfismos gênicos associados à SAM também estavam presentes em pacientes com SIMP-TS. **Métodos:** DNA de dez pacientes pediátricos com SIMP (grupo caso) e dez pacientes COVID-19 sem SIMP (grupo controle) foram genotipados por análise de PCR em tempo real (tecnologia TaqMan[®]) para polimorfismos de nucleotídeo único (SNPs) em quatro genes selecionados associados com SAM: *perforina 1 (PRF1)*, *granzima B (GZMB)*, *sintaxina 11 (STX11)* e *proteína de ligação de sintaxina 2 (STXBP2)*. A análise dos SNPs foi realizada utilizando o modelo aditivo, dominante e recessivo. **Resultados:** Uma frequência significativamente maior de um SNP (alelo selvagem C em rs6573910) no gene *GZMB* foi observada pelos modelos aditivo e dominante no grupo SIMP quando comparado aos controles. Além disso, uma significância limítrofe foi observada para o alelo G em rs7764017 do gene *STX11* no grupo

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group in the additive model. **Conclusions:** This study indicated the presence of two polymorphisms in genes associated with macrophage activation syndrome (*GZMB* and *STX11*) in patients who developed PIMS-TS. If the presence of these SNPs is validated in a larger number of PIMS-TS cases, they can be used as potential biomarkers for early identification of pediatric patients with a higher probability of developing PIMS-TS associated with SARS-CoV-2 infection.

Keywords: COVID-19, pediatric inflammatory multisystem syndrome, macrophage activation syndrome, hemophagocytic lymphohistiocytosis, single nucleotide polymorphism, polymorphism.

SIMP pelo modelo aditivo. **Conclusões:** Nosso estudo indicou a presença de dois polimorfismos em genes associados à SAM (*GZMB* e *STX11*) em pacientes que desenvolveram SIMP-TS. Uma vez validada a presença desses SNPs em um número maior de casos de SIMP-TS, eles podem ser usados como potenciais biomarcadores para a identificação precoce de pacientes pediátricos com maior probabilidade de desenvolver SIMP-TS associado à infecção por SARS-CoV-2.

Descritores: COVID-19, síndrome multissistêmica inflamatória pediátrica, síndrome de ativação macrofágica, linfohistiocitose hemofagocítica, polimorfismo de nucleotídeo único, polimorfismos.

Introduction

In the second half of April 2020, a new syndrome potentially associated with SARS-CoV-2 infection that affected children and adolescents was described in the United Kingdom,¹ Italy,² and New York,³ followed by reports worldwide. This syndrome, called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS), was shown by epidemiological studies to occur approximately 1 month after peak COVID-19 incidence. The clinical and serological characteristics of this syndrome are similar to diseases such as Kawasaki-like disease, toxic shock syndrome, and macrophage activation syndrome (MAS).⁴⁻⁶

Although the pathophysiology of PIMS-TS is not well understood and the clinical symptoms are heterogeneous at presentation, it has been characterized as a delayed immune phenomenon associated with a hyperinflammatory state, a common feature of the diseases mentioned above.

MAS involves aggressive and life-threatening conditions; immune system activation can be triggered by several types of infectious and/or rheumatological conditions and can also occur in patients with Kawasaki syndrome. Interestingly, patients with COVID-19 show similar inflammatory symptoms to secondary hemophagocytic lymphohistiocytosis (sHLH)/MAS, particularly those associated with specific rheumatological conditions, and isolated cases of COVID-19 have been reported to develop MAS. In addition, specific polymorphisms associated with MAS, such as those in the *pentraxin 3* gene, have been associated with respiratory complications, including those in adults with COVID-19. Taken together, these data provide strong evidence that SARS-CoV-2 infection can lead to MAS. Hence,

understanding of the clinical and biological similarities between patients with PIMS-TS and sHLH/MAS could help clarify the intricate immunoinflammatory response involved in these diseases.

In the present study, we tested whether polymorphisms in four genes, *PRF1*, *GZMB*, *STX11*, and *STXBP2*, which are associated with sHLH/MAS, occurred in patients with PIMS-TS. Detecting these polymorphisms could help identify pediatric patients susceptible to PIMS-during the early phase of the disease.

Materials and methods

Study population

Peripheral blood samples from 10 pediatric patients (aged 2-12 years) with confirmed PIMS-TS (case group) were collected from the pediatric Hospital Pequeno Príncipe (Curitiba, PR, Brazil) between October 2020 and April 2021. The samples were collected during hospitalization after obtaining informed parental consent. This study was approved by the National Research Ethics Committee (4.572.430/2020). The definition of PIMS-TS was based on the WHO criteria.⁷ A real-time reverse transcriptase-polymerase chain reaction assay for SARS-CoV-2 (nasopharyngeal swabs) was performed for all 10 patients during ICU hospitalization and all were negative. *Post-mortem* lung biopsies of 10 adults who died from severe COVID-19 were used as a control group (non-PIMS-TS COVID-19 group). These patients were part of a previous study (National Research Ethics Committee approval 3.944.734/2020) conducted by our group.⁸ The clinical and laboratory data for the PIMS-TS and non-PIMS-TS COVID-19 patients are presented in Table 1.

DNA isolation and genotyping

Biopur Mini Spin Plus Kit (Mobius Life Science, Curitiba, PR, Brazil) was used to isolate DNA from peripheral blood samples of the case group. In the control group, DNA was isolated from formalin-fixed paraffin-embedded tissue using a commercially available DNA isolation kit (Qiagen, Venlo, Netherlands). Single nucleotide polymorphism (SNP) selection was based on reported associations with sHLH/MAS^{9,10} and was further verified using the SNPinfo Web Server.¹¹ Four genes and 8 SNPs were selected: *perforin 1* (*PRF1* - rs10999426, rs885821, rs885822, and rs35947132); *granzyme B* (*GZMB* - rs6573910); *syntaxin 11* (*STX11* - rs7764017), and *syntaxin binding protein 2* (*STXBP2* - rs6791;

rs2303115). The patients' purified DNA was amplified by real-time reverse transcriptase-polymerase chain reaction using a TaqMan fluorescence system (Applied Biosystems, Waltham, MA, USA) according to manufacturer instructions. Family genetic analysis was used to visualize the results. Using an additive model made it possible to observe the frequency distribution between the groups. Specific groupings were also organized into dominant and recessive family grouping models.

Statistical analysis

Nominal and non-nominal variables (genotyping) were expressed as number and frequency/percentages and by median and interquartile range, respectively.

Table 1

Clinical and laboratory baseline characteristics of the PIMS-TS COVID-19 (case) and non-PIMS-TS COVID-19 (control) groups

Variables	PIMS-TS (n=10)	non-PIMS-TS COVID-19 (n=10)
Age ^a	9.5 (2.0-12.0)	80.5 (46.0-87.0)
Sex ^b		
Male	5 (50.0)	6 (60.0)
Female	5 (50.0)	4 (40.0)
Clinical aspects (symptoms)		
Fever	7/10	1/10
Abdominal pain	4/10	–
Skin rash	3/10	–
Vomiting	3/10	0/10
Bipalpebral edema	2/10	–
Myocarditis	2/10	–
High blood pressure	–	7/10
Obesity (BMI>30)	–	1/10
Laboratory test results ^a		
CRP (mg/L)	222.500 (9.200-281.000)	126.800 (52.000-171.400)
D-Dimer (ng/mL)	1187.05 (93.00-2230.67)	705.00 (425.00-6544.00)
Ferritin (ng/mL)	133.300 (13.9-133.5)	–
Troponin (pg/ml)	5.0 (5.0-90.0)	15200 (9.000-24.000)
Platelets (mm ³)	141000 (66000-314000)	121500 (111000-311000)
Lymphocytes (mm ³)	6349.50 (510.00-30110.00)	718.50 (423.00-990.00)

BMI = Body mass index; PIMS-TS = Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2.

^a Median/Interquartile range (Q1-Q3); ^b Absolute number (percentage); – Not available or detected.

In the additive model, significance was determined by logistic regression analysis, while Fisher's exact test or Pearson's chi-square test was used in the dominant and recessive models. Bonferroni correction was used for multiple testing, with $p < 0.002$ considered significant. The data were analyzed using IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA).

Results

Ten cases and 10 controls were genotyped for polymorphisms in the 4 selected genes associated with sHLH/MAS. In the additive model, only the polymorphism for *GZMB*, a wild C allele in rs6573910

(C/T), was significantly ($p = 0.028$) more frequent in the PIMS-TS group (Table 2). For the *STX11* gene in the additive model, the polymorphic GG genotype in rs7764017 SNP was present only in the PIMS-TS group, with borderline significance ($p = 0.076$). The G allele of the same gene was more frequently in the PIMS COVID-19 group by the dominant model (Table 3), however also with no statistical significance ($p = 0.087$; OR = 1.6 and CI = 1.0-1.6). Of relevance to note, that after Bonferroni correction, all the significant p-values observed in this initial analysis were lost, as the adjusted significant p-value for these genotypic associations were set as < 0.002 .

Table 2

Genotypic SNP analysis of the *PRF1*, *GZMB*, *STX11* and *STXBP2* genes in 2 groups of patients using the additive model

Gene - reference SNP ^a Allele variation [1/2]	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	p-value
<i>PRF1</i> - rs10999426 A/G	AA	AG	GG	
PIMS-TS	0 (0.0)	6 (60.0)	4 (40.0)	0.303
non-PIMS-TS COVID-19	0 (0.0)	9 (90.0)	1 (10.0)	
<i>PRF1</i> - rs885821 A/G	AA	AG	GG	
PIMS-TS	0 (0.0)	1 (10.0)	9 (90.0)	0.273
non-PIMS-TS COVID-19	1 (10.0)	3 (30.0)	6 (60.0)	
<i>PRF1</i> - rs885822 A/G	AA	AG	GG	
PIMS-TS	3 (30.0)	7 (70.0)	0 (0.0)	0.356
non-PIMS-TS COVID-19	1 (10.0)	8 (80.0)	1 (10.0)	
<i>PRF1</i> - rs35947132 A/G	AA	AG	GG	
PIMS-TS	0 (0.0)	0 (0.0)	10 (100.0)	0.474
non-PIMS-TS COVID-19	0 (0.0)	2 (20.0)	8 (80.0)	
<i>GZMB</i> - rs6573910 C/T	CC	CT	TT	
PIMS-TS	6 (60.0)	3 (30.0)	1 (10.0)	0.028
non-PIMS-TS COVID-19	1 (10.0)	3 (30.0)	6 (60.0)	
<i>STX11</i> - rs7764017 A/G	AA	AG	GG	
PIMS-TS	3 (30.0)	3 (30.0)	4 (40.0)	0.076
non-PIMS-TS COVID-19	6 (60.0)	4 (40.0)	0 (0.0)	
<i>STXBP2</i> - rs6791 A/G	AA	AG	GG	
PIMS-TS	2 (20.0)	3 (30.0)	5 (50.0)	0.400
non-PIMS-TS COVID-19	1 (10.0)	6 (60.0)	3 (30.0)	
<i>STXBP2</i> - rs2303115 A/G	AA	AG	GG	
PIMS-TS	4 (40.0)	4 (40.0)	2 (20.0)	0.214
non-PIMS-TS COVID-19	1 (10.0)	4 (40.0)	5 (50.0)	

^a SNP identifier based on NCBI; 1 = wild type allele; 2 = mutated allele. PIMS-TS: Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2. Genotypes expressed as number (%); Logistic regression, $p < 0.05$ = significant. After the Bonferroni test, $p < 0.002$ = significant.

Discussion

PIMS-TS is a serious disease that affects pediatric patients who manifest, among other symptoms, hyperinflammatory shock that can involve several organs.¹² Specifically, children present with high fever, rash, conjunctivitis, peripheral edema, and gastrointestinal symptoms. The latest bulletin from the Brazilian Ministry of Health¹³ reported 319 cases of PIMS-TS (aged 0-19 years), with 23 deaths recorded. Specifically, 15 cases of PIMS-TS and 3 deaths were reported at Hospital Pequeno Principe between March 2020 and June 2021 (6 boys and 9 girls; mean age 9.7 years). In our sample of 10 PIMS-TS cases, the sex distribution was equal and the mean patient age was 9.5 years. The patients developed the commonly reported symptoms, with fever, abdominal pain, vomiting, and skin rash being the most frequent (30-70% of the patients).

MAS is a severe, potentially life-threatening condition characterized by excessive activation of well-differentiated macrophages. It results in fever, hepatosplenomegaly, lymphadenopathy, severe cytopenia, serious liver disease, coagulopathy, and neurologic involvement.^{14,15} This condition is usually seen in systemic-onset juvenile idiopathic arthritis and rarely manifests with other subtypes of the disease.¹⁶ A variety of triggering factors, including viral infections, have been implicated in the pathogenesis of MAS associated with systemic-onset juvenile idiopathic arthritis.¹⁷ MAS has also been associated with cytokine storm in COVID-19 patients, which supports its association with sHLH in PIMS-TS. In about 50% of patients with sHLH, the underlying genetic defect is caused by mutations in genes of the apoptotic program (such as those assessed in the present study), which leads to death in target cells.¹⁸ The similar clinical symptoms and the physiological processes among these diseases and PIMS-TS, as well as the presence of polymorphisms in genes associated with sHLH/MAS, provided the rationale for our study. Among polymorphisms in the 4 investigated genes, 2 were identified more frequently or only in the PIMS-TS group: rs6573910 (C/T) in *GZMB* and rs7764017 (A/G) in *STX11*.

The *GZMB* gene is located in the 14q12 chromosome region and encodes a member of the granzyme subfamily of proteins, part of the peptidase S1 family of serine proteases.¹⁹ The encoded preproprotein is secreted by natural killer cells and cytotoxic T lymphocytes, which proteolytically process it to generate the active protease (granzyme B) that

induces apoptosis. The granzyme B also processes cytokines and degrades extracellular matrix proteins, playing an essential role in chronic inflammation and wound healing. Expression of this gene, which occurs mostly in the bone marrow, has been reported as elevated in patients with cardiac fibrosis and pulmonary cystic fibrosis.¹⁹⁻²² Interestingly, Ramcharan et al. (2020) described cardiac findings and short-term outcomes in children with PIMS-TS that may be due to post-viral immune reactions²². Furthermore, pulmonary fibrosis is a post-COVID-19 event related to remodeling and inflammatory processes during the course of the disease.^{23,24} Hence, these findings reinforce the involvement of granzyme B in inflammatory pathways and fibrosis. Granzyme B has also been associated with sHLH/MAS. Based on this evidence, this protease could be involved in PIMS-TS due to its similarities to MAS. Rs6573910 (C/T) is a 2KB upstream intronic variant in *GZMB*. The reported minimum allele frequency in the 1000G Database is 0.3017 for the T allele and 0.6983 for the C allele.²³ In our PIMS-TS sample, the frequency detected for this allele was 0.2500 for the T allele and 0.7500 for the C allele. According to the additive model, the wild C allele in rs6573910 (C/T) in *GZMB* was also more frequent in the PIMS-TS group than the control group ($p = 0.028$). Therefore, even considering that the initial significance of this association was not maintained after Bonferroni correction, the C allele for this SNP could be a risk factor for PIMS-TS.

The *STX11* gene is located at 6q24.2 and consists of 9 exons. Like *GZMB*, the translated protein, syntaxin, is highly expressed in the bone marrow. This protein has been implicated in intracellular transport vesicles and may regulate protein transport in the endosome and trans-Golgi network. Mutations in this gene have been associated with primary hemophagocytic lymphohistiocytosis.²⁵ In this gene, we observed a polymorphism in rs7764017 (A/G) in *STX11*. In the 1000G database, the minimum allele frequency reported in rs7764017 (A/G) is 0.3842 for the wild A allele and 0.6158 for the polymorphic G allele.²⁶ In our patients, the observed frequency was 0.4500 for the A allele and 0.5500 for the G allele. In the additive model, the GG genotype of this SNP was observed only in the PIMS-TS group ($p = 0.076$). Because rs7764017 is located in the intron and is classified as a non-coding transcript variant,²⁶ this SNP may not impact the translation process of the *STX11* gene. However, it cannot be ruled out that it

Table 3

Genotypic SNP analysis of the *PRF1*, *GZMB*, *STX11* and *STXBP2* genes in 2 groups of patients using the dominant and recessive models

Gene - reference SNP ^a and allele variation	Models		PIMS-TS (n=10)	non-PIMS-TS COVID-19 (n=10)	p-value
<i>PRF1</i> rs10999426 A/G	Dom A	AA+AG	0 (0.0)	0 (0.0)	N/A
		GG	0 (0.0)	0 (0.0)	
	Rec A	GG+AG	0 (0.0)	0 (0.0)	N/A
		AA	0 (0.0)	0 (0.0)	
<i>PRF1</i> rs885821 A/G	Dom A	AA+AG	1 (10.0)	4 (40.0)	0.303 ^b
		GG	9 (90.0)	6 (60.0)	
	Rec A	GG+AG	10 (100.0)	9 (90.0)	1.000 ^c
		AA	0 (0.0)	1 (10.0)	
<i>PRF1</i> rs885822 A/G	Dom A	AA+AG	10 (100.0)	9 (90.0)	1.000 ^c
		GG	0 (0.0)	1 (10.0)	
	Rec A	GG+AG	7 (70.0)	9 (90.0)	0.582 ^b
		AA	3 (30.0)	1 (10.0)	
<i>PRF1</i> rs35947132 A/G	Dom A	AA+AG	0 (0.0)	0 (0.0)	N/A
		GG	0 (0.0)	0 (0.0)	
	Rec A	GG+AG	0 (0.0)	0 (0.0)	N/A
		AA	0 (0.0)	0 (0.0)	
<i>GZMB</i> rs6573910 C/T	Dom C	CC+CT	9 (90.0)	4 (40.0)	0.057 ^c
		TT	1 (10.0)	6 (60.0)	
	Rec C	TT+CT	4 (40.0)	9 (90.0)	0.057 ^c
		CC	6 (60.0)	1 (10.0)	
<i>STX11</i> rs7764017 A/G	Dom A	AA+AG	6 (60.0)	10 (100.0)	0.087 ^c
		GG	4 (40.0)	0 (0.0)	
	Rec A	GG+AG	7 (70.0)	4 (40.0)	0.370 ^b
		AA	3 (30.0)	6 (60.0)	
<i>STXBP2</i> rs6791 A/G	Dom A	AA+AG	5 (50.0)	7 (70.0)	0.650 ^b
		GG	5 (50.0)	3 (30.0)	
	Rec A	GG+AG	8 (80.0)	9 (90.0)	1.000 ^b
		AA	2 (20.0)	1 (10.0)	
<i>STXBP2</i> rs2303115 A/G	Dom A	AA+AG	8 (80.0)	5 (50.0)	0.214 ^b
		GG	2 (20.0)	5 (50.0)	
	Rec A	GG+AG	6 (60.0)	9 (90.0)	0.350 ^b
		AA	4 (40.0)	1 (10.0)	

^a SNP identifier based on NCBI dbSNP; Dom: Dominant model; Rec: Recessive model; Genotypes expressed as number (%); N/A: not applicable; ^b Pearson chi-square. ^c Fisher's exact test. After the Bonferroni test, $p < 0.002$ = significant.

influences the splicing process of syntaxin mRNA and affects its translation.

Taken together, this preliminary study found 2 SNPs that are associated with sHLH/MAS, are present in PIMS-TS patients, and may have an etiologic role in the syndrome. Additional studies with a larger number of PIMS-TS cases and pediatric controls without PIMS-TS are necessary for independent validation of these findings and to determine these SNPs' potential as biomarkers for PIMS-TS.

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Bioequivalence of 2 formulations of montelukast (5 mg chewable tablet) in the fasting state: an open-label randomized, single-dose, 2-period, crossover study

Estudo de bioequivalência entre duas formulações de comprimidos mastigáveis de Montelukaste de 5 mg em condições de jejum: um estudo randomizado, de dose única, aberto, dois períodos, cruzado

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ABSTRACT

Introduction: Montelukast, a selective and active leukotriene receptor antagonist, is one of the most common agents in asthma treatment for both adults and children. **Objective:** To assess whether the pharmacokinetic profiles of two formulations of montelukast were similar after a single 5 mg dose (chewable tablets), administered orally under fasting conditions, sampling blood before and 36 hours after administration. **Methods:** This was a randomized, 2-sequence, 2-period, crossover study of 2 chewable tablet formulations of the drug: Singulair®, provided by Merck Sharp and Dohme Farmacêutica Ltda. (reference), and generic montelukast, manufactured by Eurofarma Laboratórios S.A. (test). Plasma samples obtained from 35 participants were analyzed for montelukast through high-performance liquid chromatography coupled to tandem mass spectrometry, with montelukast-d6 as the internal standard. Peak montelukast concentrations were 299.313 (SD, 11.039) ng/mL for the reference formulation and 279.803 (SD, 10.085) ng/mL for test formulation. **Results:** Statistical analysis showed no significant differences between AUC_{0-36h} , AUC_{0-inf} or C_{max} between formulations, with the following test/reference ratios: 102.458 for AUC_{0-36h} , 102.522 for AUC_{0-inf} and 93.490 for C_{max} . No serious adverse events were reported during the trial. Our results demonstrated the bioequivalence of Singulair® and Eurofarma's generic montelukast.

RESUMO

Introdução: O montelukaste, um antagonista seletivo e ativo dos receptores de leucotrienos, é um dos agentes mais comumente usados na prática clínica no tratamento da asma, tanto em adultos quanto em crianças. **Objetivo:** Avaliar se os perfis farmacocinéticos de duas formulações de montelukaste eram semelhantes após uma dose única de comprimidos mastigáveis de 5 mg, administrados por via oral em jejum, coletando amostras de sangue desde antes da administração até 36 horas depois disso. **Métodos:** Estudo comparativo randomizado, 2 sequências e 2 períodos, cruzado, de dois medicamentos em comprimidos mastigáveis: Singulair®, fornecido pela Merck Sharp e Dohme Farmacêutica Ltda. (referência) e Montelukaste 5 mg, fabricado pela Eurofarma Laboratórios S.A. (teste). Amostras de plasma obtidas de 35 indivíduos elegíveis foram analisadas para montelukaste por cromatografia líquida de alta eficiência acoplada a espectrometria de massa em tandem, tendo Montelukaste-d6 como padrão interno. As concentrações máximas de montelukaste foram 299,313±11,039 ng/mL para referência e 279,803±10,085 ng/mL para formulação de teste. **Resultados:** A análise estatística não mostrou diferenças significativas para AUC_{0-36h} , AUC_{0-inf} e nem para C_{max} entre as formulações, apresentando as razões Teste/Referência: 102,458 para AUC_{0-36h} , 102,522 para AUC_{0-inf} e 93,490 para C_{max} . Nenhum evento adverso grave foi relatado.

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Conclusions: Our results revealed that the new generic tablets are clinically safe and can be used interchangeably with the brand name product. Eurofarma's montelukast offers a safe, effective, and cheaper treatment option for people with asthma or allergic rhinitis.

Keywords: Montelukast, pharmacokinetics, asthma, therapeutic equivalence.

durante o estudo. De acordo com a regulamentação brasileira, o atual estudo farmacocinético demonstrou bioequivalência entre os agentes individuais e forneceu evidências de bioequivalência entre Singulair® e Montelukast fabricado pela Eurofarma.

Resultado: Os resultados mostraram que os novos comprimidos genéricos são clinicamente seguros e podem ser trocados pela marca original. O montelucaste da Eurofarma oferece uma opção de tratamento mais barata, segura e eficaz para indivíduos com asma ou rinite alérgica.

Descritores: Montelucaste, farmacocinética, asma, equivalência terapêutica.

Introduction

Leukotrienes are synthesized *in vivo* from arachidonic acid through the action of the enzyme lipoxygenase or through transcellular metabolism by cells that do not express lipoxygenase. Leukotrienes are produced and secreted by numerous cells, such as mast cells, and their pro-inflammatory effects on the respiratory system induce inflammatory cell chemotaxis, airway hyperresponsiveness, and bronchoconstriction.¹ These phenomena are less evident in the lung parenchyma and are mediated by GPCR-like receptors, stimulated preferentially by leukotrienes D4 and D5.²

Montelukast is an orally administered medicine available as chewable tablet, oral granules, or in film-coated formulations, and has been used to treat asthma, allergic rhinitis, and exercise-induced bronchoconstriction and can be administered in adults and children aged ≥ 6 months.³⁻⁵

Montelukast is a photolabile molecule produced as a powder with the following International Union of Pure and Applied Chemistry name: (R,E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid. It has been increasingly used as a treatment for seasonal and perennial allergic rhinitis.⁶ Leukotriene release causes constriction of the respiratory airways. Montelukast, which blocks the action of leukotrienes, can be used in chronic asthma prophylaxis and treatment. Thus, its pharmacological effect is due to its highly selective antagonism of D4 and E4 leukotriene receptors.⁷ Bioavailability studies have shown that food in the gastrointestinal tract does not affect bioavailability, eg, for immediate release tablets taken with a standard meal.⁸⁻¹⁰

The aim of this study was to compare the bioavailability and characterize the pharmacokinetic profile of generic montelukast relative to Singulair®. The generic product was developed to be as effective and safe as the original brand.

Methods

Study design and participant eligibility

This was a randomized 2 x 2 crossover trial of 2 medications: Singulair® and generic montelukast. Commercially available montelukast (Singulair 5 mg, Merck Sharp & Dohme, São Paulo, SP, Brazil) and montelukast (Eurofarma S.A., São Paulo, SP, Brazil), were tested. The medications were administered orally to fasting healthy male and female volunteers, who received both treatments alternately. Each medication was tested sequentially in 2 periods: a single dose was administered with fresh water at approximately 7:00 am; the volunteers remained in the hospital the preceding night, having fasted for at least 8 h. There was a washout period of 7 days between the medications.

The clinical and statistical phases of the study were conducted at the Centro Avançado de Estudos e Pesquisas Ltda (Campinas, SP, Brazil), while the analytical phase was conducted at MAGABI Pesquisas Clínicas e Farmacêuticas Ltda. (São Paulo, SP, Brazil). This study was conducted in accordance with guidelines for clinical, laboratory, and statistical procedures, Brazilian regulations for research in human subjects, and Brazilian health authority (ANVISA) bioequivalence guidelines. All participants provided written informed consent prior to enrollment. The study was approved by the Investiga

Instituto de Pesquisas Ethics Committee (CAAE 45147721.3.0000.5599). Only adults were enrolled in the trial, primarily due to the expected pharmacokinetic similarity between adults and children,¹¹ as well as to ethical concerns about unnecessarily exposing children and adolescents to treatment and blood sampling.¹²

The eligibility criteria were: men and women aged 18-55 years; non-smokers (or maximum 5 cigarettes per day); body mass index between 18.5 and 29.9 kg/m²; negative serum tests for HIV-1, HIV-2, hepatitis B, and hepatitis C; normal hemogram values; normal leukocyte and platelet counts; normal urinalysis; normal serum levels of creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, fasting glucose, and cholesterol; negative for SARS-CoV-2; in addition to normal beta-HCG labels for women. The main exclusion criteria were: a history of allergy to montelukast or related drugs; any evidence of organ dysfunction; a history of gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological, or hematological disease, diabetes, glaucoma, psychiatric disorders, psychotropic drug or alcohol abuse; the use of any drug substances in the 14 days prior to the study; participation in another clinical trial in last 6 months; recent blood donation (< 3 months), or lack of adequate venous access.

Blood collection and analysis

Blood samples (7.5 mL) were drawn into lithium heparin vacuum tubes prior to drug administration (time 0) and at 00:20 / 00:40 / 01:00 / 01:20 / 01:40 / 02:00 / 02:20 / 02:40 / 03:00 / 03:20 / 03:40 / 04:00 / 04:30 / 05:00 / 06:00 / 08:00 / 10:00 / 12:00 / 24:00 and 36:00 (h:min) after drug administration. The samples were centrifuged at 3500 rpm for 10 min at 4 °C, handled under yellow light, and the plasma was separated and stored at -70 °C in amber flasks until transfer to the analytical facility.

Sample analysis

The plasma samples were analyzed for montelukast levels using high performance liquid chromatography coupled to an API5000 mass spectrometer (Sciex, Concord, ON, Canada). Electrospray ionization detection (positive ion mode) was used to ionize the analytes and montelukast-D6, the internal standard. The samples were extracted (50 µL added to 50 µL of a 200 ng/mL montelukast-D6 solution)

through protein precipitation with methanol. After precipitation, the samples were vortex-mixed for 3 min and centrifuged at 20,800 RCF for 5 min. Five µL of the supernatant was injected into an Agilent 1200 Chromatography System (Agilent, Santa Clara, CA, USA). The samples were processed in a low light environment to avoid isomeric transformation of the drug. The chromatographic conditions for the analyte were an Agilent, Zorbax Eclipse XDB, C18, 1.8 µm (20 x 4.6 mm) column at 0.75 mL/min and, as the mobile phase isocratically pumped, a mixture of methanol/water + 23 mM formic acid (85/15; v/v). The total run time was 3.0 min. The retention times for each analyte were 1.55 min for montelukast and montelukast-D6. The mass spectrometry parameters were: dwell time 200 ms, collision energy 43 V, and collision exit energy 28.6 V for both the analyte and the internal standard. The source temperature was 600 °C; the flow rates for curtain gas, ion source gas 1, and ion source gas 2 were 10, 40, and 60 psi, respectively; and the ion spray voltage was 5500 V. Data acquisition and analysis were performed in Analyst 1.4.2. Montelukast was quantified using the multiple reaction monitoring m/z ratio (montelukast: 586.1 > 278.1; montelukast-D6: 592.4 > 278.1).

Pharmacokinetic analysis

Values for peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were taken directly from the observed concentration-time profiles. The area under the curve (AUC) for plasma concentration vs time from 0 to the last measurable concentration (AUC_{0-t}) was calculated with the linear trapezoidal rule. The AUC for 0 to infinity (AUC_{0-inf}) was calculated as $AUC_{0-t} + Ct/Kel$, where Ct is the last measurable concentration and Kel is the elimination rate constant.

Statistical analysis

For the relative bioavailability analysis, AUC_{0-t} and C_{max} were considered the primary variables. The analysis was performed in R. Component bioequivalence was assessed with analysis of variance and 90% confidence intervals for the test/reference ratio, using log-transformed data for C_{max} and AUC_{0-t} . The formulations were considered bioequivalent if the confidence interval for the ratio of the means was within the interval (0.80-1.25) or if the 90% confidence interval for the difference in means on the natural log scale was within the interval.

Results

Population

A total of 36 eligible healthy volunteers (18 male and 18 female) were enrolled in the study, but 1 woman dropped out (premature termination). Their ages ranged from 18 to 51 years, and their body mass index ranged from 19.63-29.81 kg/m².

Pharmacokinetic results

The bioanalytical method used to quantify montelukast in human plasma proved to be fast, accurate, and sensitive. The lower limit of quantification (LLOQ) for montelukast was 1.00 ng/mL. The concentration range for the calibration curves was linear from 1.00 to 1000.00 ng/mL.

The bioanalytical method was validated for precision, accuracy, recovery, specificity/selectivity, and biological matrix stability for each analyte. All results were in accordance with international bioanalytical method validation guidelines.

The mean (SD) plasma pharmacokinetic measures obtained after oral administration of a single dose of both the reference and test formulations are shown in Table 1. The mean plasma concentrations of montelukast are shown in Figure 1.

Table 2 shows the test/reference ratios and 90% confidence intervals for bioequivalence regarding AUC_{0-t} and C_{max} (log-transformed data). The statistical analysis showed no significant differences in AUC_{0-25h} or C_{max} between the formulations, demonstrating bioequivalence according to the selected criteria (90% CIs within 0.80 and 1.25). The mean AUC_{0-36h}/AUC_{0-inf} ratios were 0.990 and 0.989 for the reference and test formulations, respectively, indicating that the sampling time was adequate for both drugs.

Discussion

Our results show that both montelukast formulations had similar pharmacokinetic behavior after a single dose. The statistical analysis confirmed 90% confidence intervals for C_{max} and AUC_{0-t}, which are within the predefined limits of 80.00-125.00%, according to ANVISA and international guidelines. Wong et al. developed a chromatographic method with a faster run time but a higher LLOQ (3.0 ng/mL),⁹ while Zaid et al. found a higher LLOQ (6.1 ng/mL).¹⁰ Our LC-MS/MS bioanalysis method for drug quantification had high sensitivity and specificity, as well as high sample throughput required for pharmacokinetic, including bioequivalence, studies.

Table 1

Pharmacokinetic measurements for the reference and test formulations of montelukast after oral administration in the fasting state

Pharmacokinetic parameters	Montelukast, n=35	
	Reference Mean (±SE)	Test Mean (±SE)
C _{max} (ng/mL)	299.313±11.039	279.803±10.085
AUC _{0-36h} (ng.h.mL-1)	1954.590±84.829	1980.755±70.081
AUC _{0-inf} (ng.h.mL-1)	1974.813±86.038	2002.386±71.201
T _{max} (h)	2.490±0.145	2.914±0.174
t _{1/2} (h)	5.367±0.073	5.493±0.089

SE= standard error; AUC_{0-36h}= area under the curve for plasma concentration vs time from 0 to the last measurable concentration (36 h); AUC_{0-inf}= AUC from 0 to infinity; C_{max}= peak plasma concentration; SD= standard deviation; T_{max}= the time to reach C_{max}; t_{1/2}= elimination half-life.

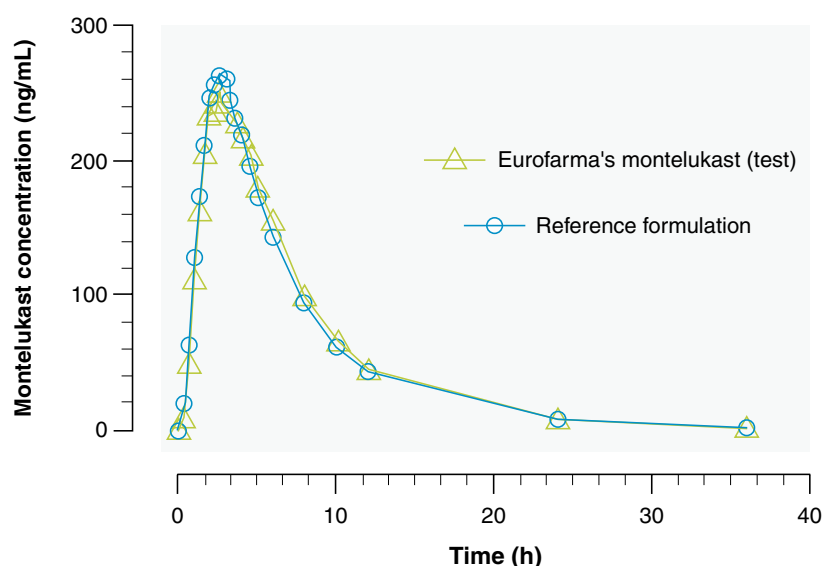


Figure 1
Mean plasma concentrations obtained in 35 participants after oral administration of Singulair (reference) and Eurofarma's montelukast (test)

Demonstrating that these montelukast formulations are bioequivalent may facilitate access to the drug. Due to the lower investment costs associated with generic or branded generic products, the introduction of Eurofarma's montelukast could have cost-effectiveness and budget implications.¹³

ANVISA and other regulatory agencies define a brand drug as a drug marketed under a proprietary, trademark-protected name, while a generic drug is

similar to the brand name drug regarding the chemical formulation of the active ingredient, dosage, strength, route of administration, quality, safety, and efficacy according to the pharmacokinetic profile, and intended use. However, generic versions of a drug can vary in quality and visual appearance, including shape, labeling, and packaging. If all of the above criteria are met, then the drugs are considered therapeutically equivalent and can be safely used interchangeably.

Table 2

Pharmacokinetic measures obtained after oral administration (5 mg) of the reference and test formulations of montelukast

Parameter	N	Ratio (%)	90% CI (%)	CV (intra) (%)	Power (%)	p-value ^a
C_{max} (ng/mL)	35	93.490	(89.260 – 97.919)	11.476	100	0.779
AUC_{0-36h} (ng.h.mL ⁻¹)	35	102.458	(98.431 – 106.649)	9.931	100	0.530
AUC_{0-inf} (ng.h.mL ⁻¹)	35	102.522	(98.517 – 106.689)	9.869	100	0.526

^a Partial sequence effect; AUC_{0-36h} = area under the curve for plasma concentration vs time from 0 to the last measurable concentration (36 h); AUC_{0-inf} = AUC from 0 to infinity; C_{max} = peak plasma concentration; CV = coefficient of variation.

All validation parameters were carried out according to International Conference on Harmonization guidelines and were within the accepted parameters, as shown in Table 1. Regarding the efficacy of the generic drug, statistical comparison of the main pharmacokinetic parameters (AUC_{0-36} , AUC_{0-inf} , C_{max} and T_{max}) showed no significant differences between test and reference tablets in any of the assessed parameters.

Several clinical studies have shown the efficacy of Singulair immediate release coated tablets for asthma treatment in both adults and children, as well as for symptom relief in allergic rhinitis. Since this study demonstrated the bioequivalence of the 2 formulations, Eurofarma's montelukast should have the same efficacy and safety profile.

Conclusions

Since this study has demonstrated the bioequivalence of 5 mg chewable tablets of generic montelukast and Singulair, we conclude that the two formulations can be used interchangeably. Statistical analysis of the AUC_{0-36} , AUC_{0-inf} and C_{max} results with analysis of variance showed that the test (Eurofarma) and reference (Singulair) products are bioequivalent, given that they release equivalent quantities of the active ingredient into systemic circulation at equivalent rates for both AUC_{0-36} and C_{max} ratios in the 80-125% interval.

These results show that the new generic tablets are clinically safe and can be used interchangeably with the original brand. Eurofarma's montelukast offers a safe, effective, and cheaper treatment option for people with asthma or allergic rhinitis.

Conflict of interest

Guilherme Araújo Pinto and Klaus Nunes Ficher work at Eurofarma Laboratórios S.A. All other authors declare that they have no conflicts of interest.

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Pork-cat syndrome as cause of anaphylactic reaction to well-cooked meat

Síndrome gato-porco como causa de reação anafilática à carne cozida

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ABSTRACT

Pork-cat syndrome is a rare clinical syndrome that can cause life-threatening reactions. Occurring in patients allergic to cat dander, it involves cross-reactivity between cat and pig serum albumin. Cat allergy usually precedes food allergies, suggesting primary sensitization to cat serum albumin. Since these proteins are thermolabile, the reaction tends to be more severe in undercooked meat. A 27-year-old woman with persistent moderate-to-severe rhinoconjunctivitis since childhood reported 2 immediate mucocutaneous reactions after eating small amounts of pork. Skin prick tests with commercial extracts showed sensitization to pork, and prick-to-prick tests confirmed sensitization to raw pork and raw beef. Specific IgE was positive for pork, and ISAC microarray also showed sensitization to Fel d 2. SDS-PAGE and IgE immunoblotting assays were performed with raw and cooked pork extract and detected in a 60 kDa band. In the immunoblotting-inhibition assays, cat serum albumin completely inhibited IgE binding to pork extract. The patient underwent 2 oral food challenges with well-cooked pork and beef, both causing an anaphylactic reaction. The patient's history and in-vivo and in-vitro tests led to a diagnosis of pork-cat syndrome with clinical cross-reactivity to another mammalian serum albumin. This case should stimulate oral food challenges with other well-cooked mammalian meats in patients with this syndrome to establish a tolerance threshold and avoid possible unexpected anaphylactic reactions.

Keywords: Cross-reactivity, serum albumin, severity, pork-cat syndrome.

RESUMO

A síndrome gato-porco é rara e ocorre em doentes alérgicos ao pêlo de gato, envolvendo reatividade cruzada entre as albuminas séricas (AS) de gato e de porco. Normalmente, a doença respiratória a pêlo de gato precede a alergia alimentar, sugerindo uma sensibilização primária à albumina sérica de gato. Uma vez que estas proteínas são termolábeis, as reações tendem a ser mais graves com carnes menos cozidas. Mulher de 27 anos com rinoconjuntivite persistente moderada a grave desde a infância que refere duas reações imediatas mucocutâneas após ingestão de pequenas quantidades de carne de porco. Os testes cutâneos por picada com extratos comerciais mostraram sensibilização à carne de porco e os testes *prick-to-prick* confirmaram sensibilização à carne de porco e de vaca cruas. A IgE específica (sIgE) foi positiva para carne de porco, e o ensaio ISAC mostrou sensibilização a Fel d 2. Foram realizados ensaios de *immunoblotting* SDS-PAGE IgE com extratos de carne de porco crua e cozidas que detectaram uma banda de 60 kDa. Nos ensaios de inibição por *immunoblotting* a albumina sérica de gato produziu uma inibição total da ligação da IgE ao extrato de carne de porco. A doente realizou duas provas de provocação oral com carne de porco e de vaca cozidas, ambas positivas com desenvolvimento de reação anafilática. A história clínica, os testes *in-vivo* e *in-vitro* levaram ao diagnóstico de síndrome gato-porco com reatividade cruzada clínica a outras albuminas séricas de mamíferos. A síndrome gato-porco é rara e pode causar reações fatais. Este caso frisa a importância da realização de provas de provocação oral com outras carnes de mamíferos bem cozidas em doentes com esta síndrome, de forma a estabelecer um limiar de tolerância e evitar possíveis reações anafiláticas inesperadas.

Descritores: Reatividade cruzada, albumina sérica, severidade, síndrome gato-porco.

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Background

Food allergies are increasing in prevalence and severity. Up to 60%¹ of the reactions in older children, adolescents, and adults are related to cross-reactivity between aeroallergens and food allergens. Primary food allergy with sensitization to food allergens can also occur, mainly in the gastrointestinal tract.^{1,2}

Cross-reactivity is an immune-mediated reaction in which IgE antibodies that are produced to recognize a specific allergen react to a homologous one, inducing an immune response. This mainly occurs with closely related allergen molecules of phylogenetically close species or in more distant species between well-preserved molecules belonging to the same protein family and having similar functions.

Pork-cat syndrome, first described by Drouet et al. in 1994,³ is a primary IgE-mediated respiratory allergy to cat dander followed by a food allergy to pork. The prevalence of this syndrome is unknown, and there are few published reports about it.⁴⁻⁷ It results from an IgE antibody against a protein with a molecular weight of approximately 67 kDa, later identified as cat albumin Fel d 2 (minor cat allergen).^{8,9} It has high homology (79.1% AA-sequence identity) with pork albumin, and the cross-reactivity between them is responsible for the symptoms. The severity of the reaction may vary from slight (mucocutaneous symptoms) to anaphylaxis. Since serum albumins are generally heat labile, the probability that well-cooked meat will cause severe reactions should be lower.¹

Case presentation

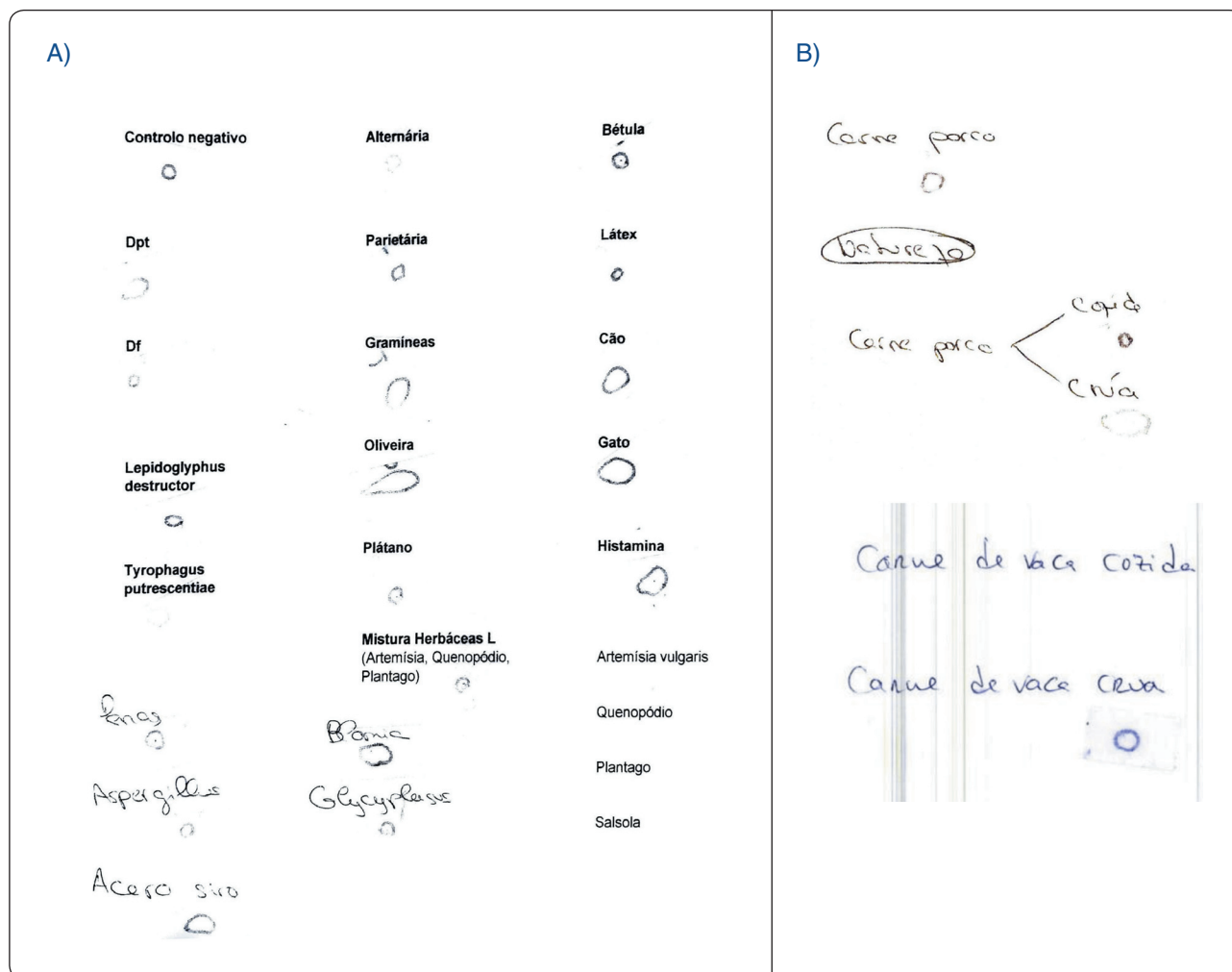
We describe the case of a 27-year-old woman born in Mozambique who has lived in Lisbon since 20 years of age. While living in Africa she ate neither pork (for religious reasons) nor beef (due to dislike). Since moving to Portugal, she has occasionally eaten small quantities of pork. In childhood, she always had outdoor cats and dogs, but in Lisbon she got an indoor dog and cat at 22 and 24 years of age, respectively. Since adolescence she has had a history of persistent moderate-to-severe rhinoconjunctivitis that worsened indoors and at night, but noticed that it had been worsening since she was 24 years of age. She denied symptoms suggestive of bronchial hyperactivity. She reported 2 mucocutaneous reactions about 30 min after ingesting small amounts of pork. The first occurred when she was 21 years of age, with labial edema and urticaria after eating a meal with pork, after which

she began to avoid it. The second episode occurred some years later, with urticaria and angioedema after accidental intake of a pork croquette (thinking it was chicken). She sometimes handled pork when helping her sister cook meals, which resulted in contact angioedema but no other symptoms. She also reported oropharyngeal pruritus related to beef, so she began avoiding that as well. She had no symptoms suggestive of cow milk allergy.

Skin prick tests with commercial extracts revealed sensitization to *Blomia tropicalis* (8 mm), *Acarus siro* (7 mm), olive pollen (13 mm), grass pollen (7 mm), cat dander (9 mm), dog dander (7 mm), and pork (5 mm). Prick-to-prick tests showed sensitization to raw pork (11 mm) and raw beef (5 mm) but were negative for well-cooked pork and beef. She also developed pruritus after 3 min and an erythematous papule 5 min after 5-minute contact with raw pork on the skin, which are compatible with immediate reaction. Contact testing with cooked pork and raw and cooked beef was negative. These results are shown in Figure 1. Serum total immunoglobulin E (IgE) was elevated (401.0 kU/L). Specific IgE was positive for cat dander (38.9 kUA/L), pork (3.47 kUA/L), *Blomia tropicalis* (2.33 kUA/L), *Acarus siro* (2.45 kUA/L), olive pollen (4.37 kUA/L), and *Dactylis glomerata* pollen (6.72 kUA/L). ImmunoCAP ISAC microarray (Thermo Fisher Scientific, Waltham, MA, USA) showed very high sensitization to Fel d 4; high-to-moderate sensitization to Fel d 1, Fel d 2, Can f 1, Can f 3, Phl p 1, and Phl p 4; and low sensitization to Equ c 1, Equ c 3, Cyn d 1, Mus m 1, and Ole e 1.

Extracts from raw and cooked pork were prepared by delipidation with acetone, homogenization in phosphate buffered saline (20% w/v) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5) with magnetic stirring, centrifugation, dialysis of the supernatants against distilled water, and lyophilization. Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE)/IgE immunoblotting assays were performed with raw and cooked pork extract, and a 60 kDa band was detected in both samples. In the immunoblotting-inhibition assays, cat albumin completely inhibited IgE binding to pork extract (Figure 2). We also performed an inhibition assay with other mammalian meat (lamb), which showed cross-reactivity with pork and cat albumin (results not shown).

The patient underwent 2 oral food challenges with cooked pork and beef, both of which were positive and with an anaphylactic reaction. In the first challenge, she developed urticaria, angioedema,

**Figure 1**

A) Skin prick tests showing sensitization to mites, pollens, and pork.

B) Prick-to-prick tests showing sensitization to raw pork and beef.

nasal blockage, serous discharge, and eye and nasal pruritus 15 min after ingesting 2 g of well-cooked pork, followed by dysphonia, dry cough, and uvula edema. She was treated with 2 doses of 0.5 cc epinephrine IM, anti-histamine, and corticosteroid, with clinical improvement in 30 min.

In the second challenge, she developed cough, dysphonia, lightheadedness, and throat tightness 10 min after ingesting 8 g of well-cooked beef, with immediate improvement after 0.5 cc of epinephrine IM.

The patient's history and *in vivo* and *in vitro* test results led to a diagnosis of pork-cat syndrome with clinical cross-reactivity to another mammalian meat.

An epinephrine auto-injector was prescribed, and the patient was advised to maintain a diet free of mammalian meat. Medical treatment led to slight improvement in rhinoconjunctivitis, but the patient reported exacerbation in closed spaces (home and office). Contrary to our advice, she made no environmental changes to reduce exposure to aeroallergens. Since then, we have heard nothing further from her.

Discussion and conclusions

Pork-cat syndrome is rare and can go unnoticed if not properly assessed. In this case, the patient had a primary sensitization to cat dander, with an evident respiratory allergy that worsened with exposure to

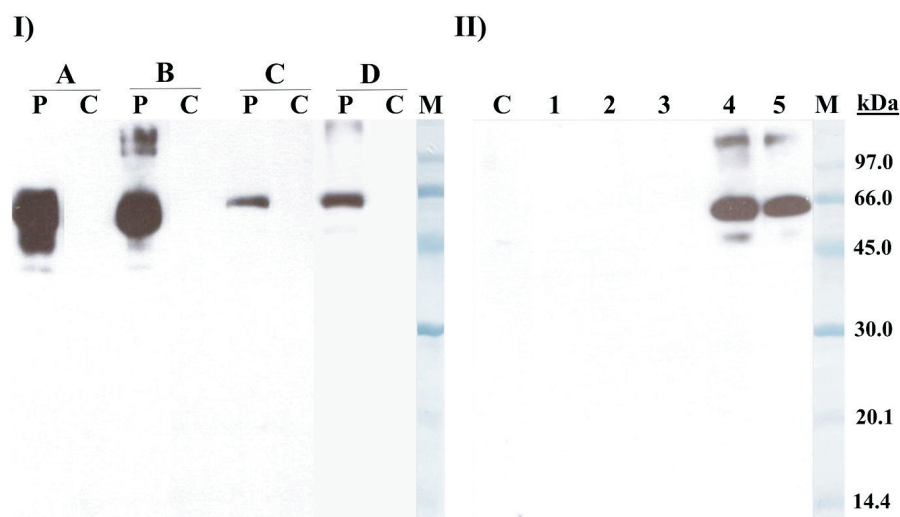


Figure 2

I) SDS-PAGE/Immunoblotting. A) Cat dander extract, B) Cat serum albumin, C) Raw pork extract, D) Cooked pork extract. Lane P: Patient serum, Lane C: Control serum (pool of sera from non-atopic individuals), Lane M: Molecular mass standard.

II) SDS-PAGE/Immunoblotting-inhibition. Solid phase: Pork extract. Lane C: Control serum (pool of sera from non-atopic individuals), Lanes 1-5: Patient serum pre-incubated with pork extract (lane 1), Cat dander extract (lane 2), Cat albumin (lane 3), Chicken ovalbumin (lane 4), and Sunflower pollen extract (lane 5), Lane M: Molecular mass standard.

high levels of the allergen (when she owned an indoor cat). She was not exposed to pork during childhood, but when she moved to Lisbon, she began to have meals that contained it. We cannot assess the role of its late introduction in food allergy development. As reported by other authors,¹⁰⁻¹³ she also had symptoms with beef, most likely due to cross-reactivity between serum albumins.

The laboratory workup proved sensitization to cat dander and pork: ISAC assay showed sensitization to Fel d 2 (cat albumin), SDS-PAGE/IgE immunoblotting detected a 60 kDa band (as shown in Hilger et al.¹²), and the immunoblotting-inhibition assays showed cross-reactivity between cat and pork albumin.

The pork food challenge established the clinical relevance of sensitization. The anaphylactic reaction to well-cooked pork was not expected because serum albumin is heat labile, losing its IgE

reactivity after exposure to high temperatures. The results of both food challenges highlight why they are considered the gold standard for diagnosing food allergies, as well as their important prognostic value, determining the tolerance threshold and risk of severe reaction.

Besides sensitization to cat dander and cat serum albumin, ISAC also showed sensitization to dog, horse, and mouse, which is explained by the cross-reactivity between serum albumin of different mammals.

Regarding aeroallergens, the patient was sensitized to *Blomia tropicalis* and *Acarus siro*, two uncommon mites in Lisbon, according to the 2011 mite map of Portugal¹³. This, plus the fact that the patient worsened when she acquired an indoor cat, suggest that the most probable cause of worsening rhinoconjunctivitis was exposure to cat dander.

Since both respiratory and food allergies are generally caused by Fel d 2 sensitization, we hypothesize specific immunotherapy to cat dander as a possible treatment for our patient. One year of subcutaneous immunotherapy with cat dander extract has led to symptom improvement in adults with rhinoconjunctivitis and asthma.¹⁴ However, Fel d 1 is the only standardized allergen in specific immunotherapy for cat dander, since this is the major cat allergen. The effects of such a therapy could be studied in patients co-sensitized to Fel d 1 and Fel d 2. Furthermore, its efficacy for pork allergy in pork-cat syndrome could also be determined, since there is currently no treatment for this syndrome.

Pork-cat syndrome is a rare clinical syndrome that can cause life-threatening reactions. Molecular analysis is fundamental to document cross-reactivity, but oral food challenges should always be performed to define the tolerance thresholds of each patient. By doing so the clinician can define which patients are at risk of anaphylaxis and develop action plans to avoid the worst clinical manifestations.

This is the third pork-cat syndrome case published to date in Portugal and it involved the most severe clinical symptoms. Our report shows the need for better awareness of this syndrome and its potentially life-threatening consequences.

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Reactive arthritis after COVID-19: a case report

Artrite reativa pós-COVID-19: relato de caso

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ABSTRACT

Complications associated with COVID-19 include renal failure, myocarditis, thrombotic events, and retinitis. However, other manifestations, such as reactive arthritis, also seem to be associated with infection and require further investigation. We report the case of a 32-year-old woman in Rio de Janeiro, RJ who developed reactive arthritis 5 days after the onset of flu-like symptoms. Laboratory tests, GeneXpert for COVID-19, and synovial fluid puncture were performed. Positive GeneXpert results for COVID-19, increased inflammatory markers, non-reactive serological markers of autoimmunity, and negative culture in synovial fluid were observed. These results ruled out both septic arthritis and rheumatoid arthritis, leading to a diagnosis of post-infectious arthritis resulting from SARS-CoV-2.

Keywords: Reactive arthritis, COVID-19, autoimmunity.

RESUMO

As complicações associadas à COVID-19 incluem insuficiência renal, miocardite, eventos trombóticos e retinite. No entanto, outras manifestações, como a artrite reativa, também parecem estar atreladas a este vírus e precisam ser melhor investigadas. O caso relatado se refere a uma paciente de 32 anos, do sexo feminino, na cidade do Rio de Janeiro (RJ), que desenvolveu um quadro de artrite reativa após 5 dias da manifestação de sintomas gripais. Foram realizados exames laboratoriais, genexpert para COVID-19 e punção do líquido sinovial. Observou-se genexpert positivo para COVID-19, aumento nos marcadores inflamatórios, marcadores sorológicos de autoimunidade não reagentes e cultura negativa no líquido sinovial. Esses resultados descartam artrite séptica, bem como artrite reumatoide, passando a ser considerado o quadro de artrite pós-infecciosa decorrente do SARS-CoV-2.

Descritores: Artrite reativa, COVID-19, autoimunidade.

Introduction

The novel coronavirus (SARS-CoV-2), which causes COVID-19, was first detected on December 31, 2019, in Wuhan City, Hubei Province of China. On January 30, 2020, the World Health Organization declared the outbreak a public health emergency of international concern. Since then, several studies have been conducted to elucidate the influence of the novel virus on the immune system.¹

The immune system defends the body against infectious agents, and such function is mediated by mechanisms of innate and adaptive immunity, which are fundamental in the response to disease-causing microorganisms. In COVID-19, a benign and self-limiting evolution of the disease depends on an efficient and balanced immune response. However, in certain cases, an exacerbated immune response

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to SARS-CoV-2 can generate more severe forms of the disease.²⁻⁴

Complications known to be associated with COVID-19 include renal failure, myocarditis, thrombotic events, and retinitis.⁵ However, because this disease is new, post-COVID-19 sequelae are not yet well understood. We report the case of a patient who developed reactive arthritis after COVID-19 infection.

Case report

A 32-year-old woman presented with pharyngitis and cough on June 8, 2022. After 5 days, she developed fever (37.8 °C), a blood pressure of 140/90 mmHg, and signs of inflammation in the right knee and right shoulder. She was admitted to the emergency room on June 11, 2022.

Laboratory tests were performed, and a radiograph of the right knee and magnetic resonance imaging (MRI) of the right knee and shoulder were obtained. The radiograph showed obliteration of the suprapatellar fat pad, suggesting a synovial reaction (Figure 1). Knee MRI showed marked joint effusion, and joint puncture was performed (Figure 2). Fluid



Figure 1
Radiograph of the right knee showing obliteration of the suprapatellar fat pad

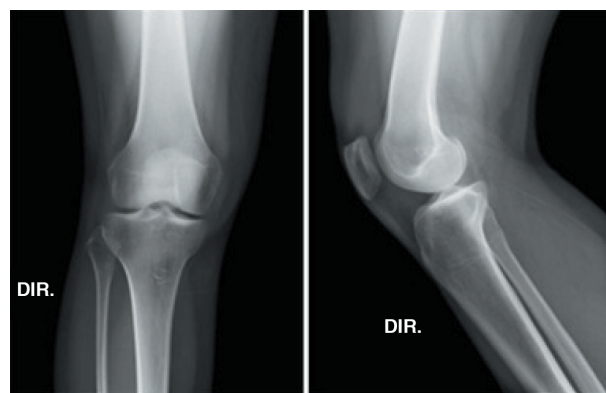


Figure 2
Magnetic resonance imaging of the right knee showing marked joint effusion

analysis showed 2,240 leukocytes per mm³, with 1,366 polymorphonuclear cells per mm³. Cultures and bacterioscopy were negative. Shoulder MRI showed effusion of the acromioclavicular and glenohumeral joints.

The patient was tested for COVID-19 using GeneXpert, which was positive, and underwent a transthoracic echocardiogram, which showed absence of vegetation. The patient tested negative for rheumatoid factor (RF), anti-streptolysin O (ASLO), antinuclear antibody, anti-cyclic citrullinated peptide antibody (anti-CCP), venereal disease research laboratory test, HIV serology, anti-hepatitis C virus, anti-hepatitis A virus, and hepatitis B surface antigen. Regarding inflammatory markers, there were increased levels of C-reactive protein (8.2 mg/dL) and a high erythrocyte sedimentation rate (41 mm). Laboratory tests showed leukocytosis (10,390/μL) with neutrophilia (7,865/μL), but normal levels of lymphocytes (1,870/μL) and basophils (0/μL). Red blood cell numbers were also normal.

The patient significantly improved after 5 days of treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), rest, and cold compresses. This clinical presentation was compatible with post-viral reactive arthritis.

Discussion

Septic arthritis is an infection of the synovial fluid caused by pyogenic bacteria.⁶ The microorganism directly invades the joint and can be identified through joint aspiration for synovial fluid analysis. In the case presented in this report, synovial fluid cultures were

negative for bacteria and fungi. Laboratory tests for syphilis, HIV, and hepatitis were also negative, which ruled out the possibility of a sexually transmitted infection. In this context, considering that the patient responded to conservative treatment, the possibility of septic arthritis was also ruled out.

Rheumatoid arthritis (RA) is another known cause of joint inflammation, but several aspects make this diagnosis less likely. The patient had no previous history of joint swelling, and serological tests for RF and anti-CCP were both negative. In addition, the most frequently affected joints in RA are the peripheral synovial joints, such as the metacarpal and metatarsophalangeal joints, followed by the ankles and wrists⁷, which were not verified in this case.

Rheumatic fever, in which arthritis is a common manifestation that classically evolves in an asymmetric and migratory way⁸, was also considered. However, the ASLO test was negative, and the transthoracic echocardiogram showed absence of vegetation, thereby ruling out the hypothesis of rheumatic fever.

Postinfectious arthritis or reactive arthritis can be defined as sterile arthritis after an infectious condition⁹, in which an exacerbated immune response seems to be involved in the pathophysiology of the disease.¹⁰ Some studies, although few, have investigated the role of SARS-CoV-2 in acute and chronic arthritis and pointed out similarities between the immune mechanism triggering COVID-19-related arthritis and the pathogenic mechanism of reactive arthritis¹¹⁻¹³, corroborating our hypothesis.

In the present case, the diagnosis of reactive arthritis after COVID-19 infection was considered. Reactive arthritis is typically described as monoarticular or oligoarticular involvement of large joints of the lower limbs after urogenital or gastrointestinal infections.¹⁴ However, it can also occur atypically in other infections.¹⁵ In this case, the characteristics of the affected joints met the diagnostic criteria for reactive arthritis. It should be noted that approximately 50% of patients with reactive arthritis may also have arthritis in the upper limbs, a condition also presented by the patient in question.¹⁶ In addition, the patient had leukocytosis with neutrophilia, increased CRP levels, and negative bacterioscopy and synovial fluid cultures, which also support the diagnosis of postinfectious arthritis.

Treatment for reactive arthritis was started on the first day of hospitalization with the NSAID ketoprofen 50 mg orally twice a day. The patient showed clinical

improvement and, after 5 days, was fully recovered and able to walk again. She was discharged from hospital with orientation to attend physical therapy sessions. One month after discharge, the patient attended an outpatient consult showing full recovery of joint mobility.

Given the limited number of publications on the subject and the patient's clinical condition, the positive molecular test for COVID-19, the negative synovial fluid cultures for bacteria and fungi, the negative serological results for autoimmune markers, and the absence of other possible diagnoses, reactive arthritis after infection with SARS-CoV-2 was considered as a diagnosis of exclusion.

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Hemophagocytosis in peripheral blood culture preceding hemophagocytosis in bone marrow biopsy in secondary hemophagocytic syndrome

Hemofagocitose em cultura de sangue periférico precedendo hemofagocitose em biópsia de medula óssea na síndrome hemofagocítica secundária

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ABSTRACT

Hemophagocytic syndrome, which is caused by dysregulation of the immune system, is characterized by excessive macrophage activation, resulting in phagocytosis of normal blood cells in the liver, spleen, and bone marrow. It can be primary (genetic) or secondary (acquired). In adults, it is almost always secondary, with infections, neoplasms, and autoimmune diseases as frequent triggers. The main manifestations of this syndrome are prolonged fever and hepatosplenomegaly. Currently, diagnosis is confirmed through finding hemophagocytosis in a bone marrow biopsy. However, it has been reported that bone marrow biopsy results are still normal on the first day the syndrome manifests. Here we report observing hemophagocytosis in cultured peripheral blood cells from a 29-year-old patient prior to finding hemophagocytosis in bone marrow biopsy. The patient had various infections and a poor general condition, which did not improve after treating the infections. The laboratory findings allowed early treatment of hemophagocytic syndrome and the patient improved. We describe our technique in detail so it can be reproduced, and we provide a non-systematic review of the literature on the syndrome.

Keywords: Hemophagocytic lymphohistiocytosis, hyperferritinemia, persistent infection.

RESUMO

A síndrome hemofagocítica é determinada por desregulação do sistema imunológico, caracterizada por ativação excessiva de macrófagos, resultando em fagocitose de células sanguíneas normais no fígado, baço e medula óssea. Pode ser primária (genética) ou secundária (adquirida). Em adultos quase sempre é secundária, tendo infecções, neoplasias e doenças autoimunes como frequentes desencadeadores. Entre as principais manifestações da síndrome estão febre prolongada e hepatoesplenomegalia. O diagnóstico até o momento é confirmado pelo achado de hemofagocitose em biópsia de medula óssea. Entretanto, é descrito que a biópsia de medula óssea é normal nos primeiros dias de manifestações da síndrome. O presente relato tem como objetivo mostrar a observação de hemofagocitose em cultura de células de sangue periférico de paciente de 29 anos precedendo a hemofagocitose em biópsia de medula óssea. A paciente apresentava diferentes infecções, com grave comprometimento do estado geral e sem melhora com o tratamento das infecções. O achado laboratorial permitiu o tratamento precoce da síndrome hemofagocítica e a melhora da paciente. No presente relato a técnica utilizada está descrita detalhadamente para que possa ser reproduzida, além de ser apresentada uma revisão não sistemática da literatura sobre a síndrome.

Descritores: Linfo-histiocitose hemofagocítica, hiperferritinemia, infecção persistente.

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Introduction

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome is characterized by increased levels of pro-inflammatory cytokines, with an excessive activation of macrophages that leads to phagocytosis of normal blood cells in the liver, spleen, and bone marrow.

The main symptoms of HLH are persistent fever above 38.5 °C and hepatosplenomegaly, but general (malaise, weakness), hematological (coagulopathy, petechiae, purpura, ecchymosis, epistaxis), liver, splenic, gastrointestinal (hematemesis, ascites, diarrhea, vomiting, abdominal pain, lower gastrointestinal bleeding), skin (erythematous rash, edema, subcutaneous nodules), respiratory (cough, dyspnea, respiratory failure), and genitourinary (nephrotic syndrome, renal failure) manifestations may also be present, as well as central nervous system (altered mental status, seizures, encephalopathy, coma) and psychiatric disorders (mood swings, delirium and psychosis).¹⁻³ HLH should be investigated in cases of febrile cytopenia, as a delayed diagnosis may lead to multiorgan impairment and increased mortality.

The most common laboratory alterations in HLH are cytopenias (anemia, thrombocytopenia, neutropenia), hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis, decreased T and natural killer (NK) cell function, and elevated levels of the soluble interleukin-2 receptor (sIL-2R or CD25).^{2,3} Other alterations may include elevated inflammatory markers (C-reactive protein and erythrocyte sedimentation rate); elevated levels of lactate dehydrogenase; cerebrospinal fluid pleocytosis; elevated levels of liver transaminases, total bilirubin, urea, and creatinine; normal or slightly elevated levels of ammonia; hypoalbuminemia; and hyponatremia.^{2,3}

The causes of HLH may be primary or secondary, both with high mortality. Primary HLH is related to genetic mutations in the *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *SH2D1*, *XIAP*, *RAB27A*, *LYST*, *AP3B1*, *NLR4*, *MAGT1*, *ITK*, and *CD27* genes and is more common in children up to 18 months of age, but may occur at any age. The incidence of primary HLH ranges from 1:50,000 to 1,000,000 live births, and both sexes are equally affected.³ The etiology of secondary HLH varies greatly according to different places: infectious etiologies are more common in the USA, France, Spain, and South Korea, whereas

neoplastic etiologies are more common in Italy, China, Japan, and Taiwan.¹ Only a few cases of HLH have been described in South America.

TCD8+ and NK cells normally release perforin (a protein that forms pores on the target cell surface) and promote granzyme activation (proteins that trigger apoptosis).^{3,4} For this process to occur normally, perforin and granzymes need to be synthesized, transported, packaged in cytolytic granules, and secreted by TCD8+ and NK cells. Mutations in genes transcribing the proteins involved in these 4 processes cause primary hemophagocytic syndrome as a result of inability to clear the antigenic stimulus, which results in a permanently activated immune response and a persistent increase in the pro-inflammatory cytokines tumor necrosis factor α , interferon γ , interleukin (IL)-1 β , IL-2, IL-6, IL-12, IL-16, and IL-18.³ Elevated anti-inflammatory response has also been observed, particularly of IL-10; however, this compensatory response is not enough to suppress excessive macrophage activation.³ The microenvironment of elevated pro-inflammatory cytokines results in persistent macrophage activation with resultant hemophagocytosis, tissue damage, organ failure, and the other manifestations of HLH.^{3,4}

The diagnostic hypothesis of HLH is achieved through the use of the HScore and HLH-2004 scores – both are used in adults and have the same sensitivity and specificity. The HLH-2004 score, which was used in the present case, is derived from studies in children.^{3,4}

HLH treatment aims to control the excessive immune response using potent immunosuppressants, human immunoglobulin, high-dose corticosteroids, and plasmapheresis, in addition to treating the underlying disease and associated multiorgan impairment.

We report the case of a 29-year-old woman in whom hemophagocytosis was first identified in peripheral blood culture and only later in bone marrow biopsy. We also describe in detail the technique used for identifying hemophagocytosis in peripheral blood.

Case report

A 29-year-old woman from Bahia, Brazil, currently living in São Paulo, Brazil, presented to the emergency room with symptoms of shortness of breath, tiredness, and productive cough for 5 weeks, with worsening of dyspnea for 1 day. She worked as a waste collector and had a history of HIV infection (she had abandoned treatment 3 years ago) and chronic kidney disease

(collapsing focal segmental glomerulosclerosis) due to HIV. She was hospitalized and suffered two seizures, which were controlled with phenytoin. She was conscious and oriented to place and time, but presented hepatomegaly (3 cm); dullness to percussion over Traube's space; palpable and painless inguinal lymph nodes on both sides (2 cm), without signs of inflammation; oxygen saturation of 96% with the use of an oxygen catheter; and tachycardia (135 bpm). During the next 6 months of hospitalization, she developed daily fever of unknown origin, chronic cutaneous herpes simplex in the perineal region, *Cytomegalovirus* (CMV) infection, candidemia, pulmonary tuberculosis, and viral myocarditis. In the sixth month of hospitalization, the patient's clinical condition significantly deteriorated, and she developed systemic inflammatory response syndrome. Laboratory tests were negative for bacteremia and showed pancytopenia, a TCD4+ cell count of 11 cells/mm³, hypertriglyceridemia, and hyperferritinemia. Considering clinical symptoms and laboratory findings, the hypothesis of hemophagocytic syndrome was raised. The HScore for Reactive Hemophagocytic Syndrome⁵ indicated a 96% chance of HLH. To verify the hypothesis of hemophagocytic syndrome, a bone marrow biopsy was performed, which showed normal histopathological results. Peripheral blood phagocyte culture was then performed, which identified the presence of hemophagocytosis in peripheral blood (Figure 1), and treatment for HLH was initiated. Three days after the blood culture, a new bone marrow biopsy was performed, now revealing hemophagocytic syndrome.

After the diagnosis was confirmed by cell culture, because other medications were unavailable at the hospital where the patient was treated and in view of the rapid and progressive worsening of her condition, pulse therapy with methylprednisolone (1 g EV for 3 days) was started. After 3 days of treatment with corticosteroids, the fever resolved, the dyspnea improved, the heart rate dropped from 135 to 80-90 bpm, and the patient no longer required oxygen therapy. After 1 week, laboratory analysis showed a decrease in ferritin levels (from 2,756 to 1,075 ng/mL) and improvement of anemia (from 6.1 to 11.9 g/dL). After 4 weeks, the patient's general condition continued to improve. She remained afebrile and eupneic, had a normal heart rate, and gained 5 kg. Hematological laboratory tests and inflammatory tests were normalized. The patient was discharge from hospital after complete resolution of HLH symptoms.

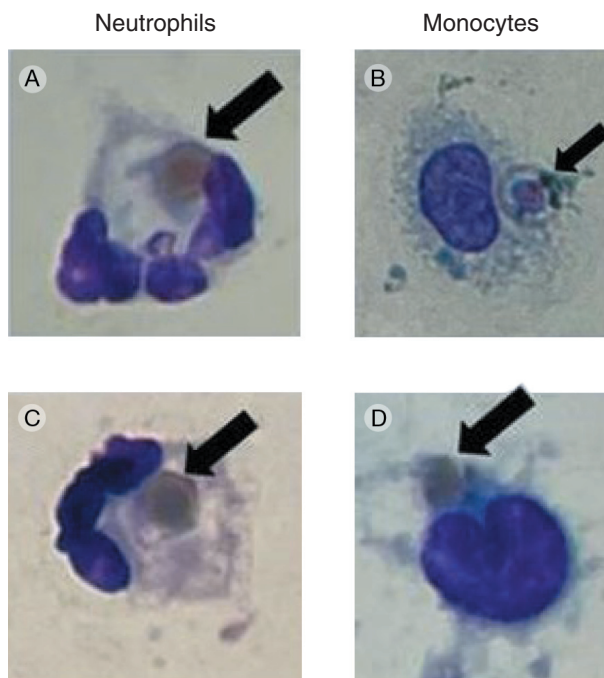


Figure 1

Peripheral blood cell culture showing hemophagocytosis by neutrophils and monocytes. The arrows indicate red blood cells phagocytosed by neutrophils (A and C) and monocytes (B and D) from peripheral blood

Discussion

The patient presented to the emergency room with high and persistent fever and hepatosplenomegaly. No symptoms were suggestive of a specific infection, and the patient responded poorly to medication. She had risk factors for developing secondary hemophagocytic syndrome, including history of HIV infection, previous tuberculosis, and treatment for CMV during the hospitalization.^{1,4,5} Such factors, in association with the patient's clinical condition (high and persistent fever, hepatosplenomegaly, adenomegaly, pancytopenia, and negative cultures), led to the hypothesis of HLH.^{1,2} This disease, although rare, has a higher incidence in people with HIV and tuberculosis, with a worse prognosis in patients with active infection.⁵

Secondary HLH can be divided into two subgroups: with infectious triggers (viral, bacterial, parasitic, fungal) and noninfectious triggers (neoplasms, autoimmune diseases, medications, and other etiologies). One third of secondary HLH cases has more than 1 cause.^{1,4} Secondary HLH has an incidence of 1:800,000 individuals and mostly affects adults and women, with a mean age at diagnosis of 50 years.¹

The patient presented different infectious causes that could trigger HLH, including HIV, tuberculosis, and CMV infection. The most common infectious causes of HLH are HIV and Epstein-Barr virus infection,^{1,4} but also include herpes, CMV, hepatitis A, B, and C, parvovirus B19, influenza, and more recently SARS-CoV-2 infection.⁶ Bacterial (*Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli* and *Rickettsia* spp.), parasitic (*Leishmania* spp., *Plasmodium* spp., *Toxoplasma* spp.), and fungal (*Histoplasma* spp.) infections have also been described.^{1,3,4,5,7}

Noninfectious causes of HLH must be excluded based on clinical condition or laboratory data, as in the present case. The main noninfectious causes of HLH are cancer (especially hematologic, such as lymphomas, leukemias, solid tumors), autoimmune diseases (systemic lupus erythematosus, Still's disease, rheumatoid arthritis, vasculitis, and inflammatory bowel disease), medications (methotrexate, nonsteroidal anti-inflammatory drugs, anticonvulsants, immunosuppressants, chemotherapy, gold salts, sulfasalazine), medical procedures (surgeries, biopsies, parenteral nutrition, hemodialysis, liver and kidney transplants), other diseases (diabetes, chronic liver diseases), and pregnancy.^{1,3}

Other causes of systemic immune dysregulation should be ruled out when diagnosing HLH, such as sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome, which present similar clinical symptoms¹⁻³ but often with a faster evolution, unlike in the present case.

The diagnostic hypothesis in this case was based on the HLH-2004 criteria: presence of fever, splenomegaly, cytopenias, hypofibrinogenemia, hypertriglyceridemia, and ferritin levels > 500 µg/L, with a score of 96% chance of having HLH. HLH should be considered when 5 of the 8 clinical criteria of the HLH-2004 score are present: (1) fever > 38.5 °C; (2) splenomegaly; (3) cytopenias affecting at least 2 lineages in the peripheral blood: hemoglobin < 9 g/L, or platelets < 100,000 cells/mm³, or leukocytes < 3,000 cells/mm³, or neutrophils < 1,000 cells/mm³; (4) hypertriglyceridemia (> 265 mg/dL) and/or hypofibrinogenemia (< 150 mg/dL); (5) hemophagocytosis in bone marrow, spleen, liver, or lymph nodes; (6) low or absent NK cell activity; (7) hyperferritinemia (> 500 ng/mL); and (8) soluble CD25 or sIL-2R > 2400 U/mL.⁸

The diagnosis is confirmed by the presence of hemophagocytosis in bone marrow biopsy. However,

biopsy results tend to be normal in the first days of symptom onset, as happened in the present case, and the absence of hemophagocytosis does not exclude HLH.⁸

Peripheral blood cell culture showed the presence or fragments of blood cells within mononuclear phagocytes and neutrophils, which led to the diagnosis of HLH before the appearance of hemophagocytosis in the bone marrow and despite the unavailability of tests such as NK cell activity and IL-2R¹¹ dosage.

Phagocytes were cultured at 37 °C, in an atmosphere of 5% CO₂, and isolated from peripheral blood by spontaneous sedimentation.⁹ The spontaneous sedimentation technique guarantees a cell suspension of approximately 77% neutrophils.¹⁰ The cell suspension also contained, in order of quantity, red blood cells, eosinophils, monocytes, and lymphocytes. This technique allows direct contact between the different cells, allowing red blood cells to be phagocytosed by other blood cells, as occurs in hemophagocytic syndrome – this led to the diagnosis of HLH in the case presented in this report.

Published studies show that bone marrow biopsy is typically normal in the first days, and early treatment is critical for patient survival. HLH is conventionally treated with etoposide, rituximab, ruxolitinib, or emapalumab,¹¹⁻¹³ and hematopoietic stem cell transplantation may be conducted in patients refractory to treatment. Because first-line therapies were unavailable and the patient's condition was worsening, pulse methylprednisolone therapy was administered. Although not a first-line therapy, the use of corticosteroids and the treatment of underlying infections allowed the control of clinical and laboratory manifestations. After treating the hyperinflammatory state, the fever completely resolved, the dyspnea improved, the patient no longer required oxygen therapy, and laboratory tests were normalized. After 4 weeks, there was complete regression of HLH, with improvement of the patient's general condition, which allowed her to be discharged from hospital for outpatient follow-up.

Conclusion

In this case report, peripheral blood culture identified hemophagocytic syndrome through neutrophilic and mononuclear phagocytes, preceding the presence of hemophagocytosis in the bone marrow. Performing peripheral blood culture may help in cases where a diagnosis of hemophagocytic syndrome is suspected but bone marrow biopsy is normal in the first days.

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Myasthenia gravis in the complex scenario of LRBA-related immune dysregulation

Miastenia gravis no cenário clínico complexo das imunodesregulações relacionadas ao gene LRBA

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ABSTRACT

Autoimmune diseases have been progressively recognized as a potential complication of primary immunodeficiency, especially for some genetic subtypes of common variable immunodeficiency. Although often associated with other autoimmune disorders, autoimmune myasthenia gravis is occasionally identified as a neuromuscular complication of primary immunodeficiency. We report the case of a Brazilian woman with common variable immunodeficiency-8 due to an *LRBA* variant, in which myasthenia gravis was identified in association with anti-acetylcholine receptor antibody. Marked clinical improvement occurred after intravenous immunoglobulin therapy.

Keywords: Myasthenia gravis, common variable immunodeficiency, immune system diseases, autoimmune diseases.

RESUMO

Doenças autoimunes foram progressivamente reconhecidas como complicações potenciais das imunodeficiências primárias, especialmente para alguns subtipos genéticos das imunodeficiências comuns variáveis. Embora se associe comumente a outras doenças autoimunes, a Miastenia gravis autoimune adquirida foi raramente associada como complicação neuromuscular de imunodeficiências primárias. É descrito neste artigo o caso de paciente brasileira do sexo feminino com diagnóstico de Imunodeficiência Comum Variável tipo 8 por variante no gene *LRBA*, na qual foi identificada Miastenia gravis em associação a anticorpos antirreceptor de acetilcolina. Ela evoluiu com marcante melhora clínica após a introdução de terapêutica com imunoglobulina endovenosa.

Descritores: Miastenia gravis, imunodeficiência de variável comum, doenças do sistema imunitário, doenças autoimunes.

Introduction

Primary immunodeficiencies have been progressively recognized in association with several autoimmune disorders, representing a complex challenge during the diagnostic work-up and follow-up treatment. Common variable immunodeficiency (CVID) is a complex clinically and genetically heterogeneous group of inherited disorders of the immune system, leading to hypogammaglobulinemia,

antibody production deficiency, variable reduction of T-cell activation/proliferation, and recurrent infections, generally with childhood onset. CVID is the most common group of primary immunodeficiency diseases.^{1,2} Autoimmune disorders are potential life-threatening complications of CVID that may involve compromised hematological, skin, gastrointestinal, neurological, and connective tissue.^{3,4} The clinical

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presentation of CVID may vary considerably with age at onset of recurrent infectious complications or autoimmune disorders, ranging from early childhood to late adulthood manifestations.¹ Lipopolysaccharide-responsive beige-like anchor (LRBA) deficiency is a rare primary immunodeficiency associated with CVID with autoimmunity-8 (CVID8), which leads to a difficult clinical scenario involving multiple autoimmunities, immune dysregulation, and high risk of malignancy, especially lymphoproliferation.^{5,6}

Myasthenia gravis is the most common acquired disorder of the neuromuscular junction, having an idiopathic or paraneoplastic autoimmune basis. Antibodies directed to the postsynaptic acetylcholine receptor and other epitopes that complement activation play a key role in its pathophysiology.^{7,8} Specific genetic markers, such as HLA polymorphisms (ie, HLA-B7, HLA-B8, HLA-DR3, and HLA-DRB1*15:01) and other concurrent autoimmune comorbidities lead to a higher risk of myasthenia gravis.⁹ Myasthenia gravis has been identified as a neuromuscular complication of primary immunodeficiencies, such as selective IgA deficiency.¹⁰⁻¹² Here we describe a rare case of autoimmune acquired myasthenia gravis as a complication of CVID8, which was treated with monthly infusions of intravenous immunoglobulin, resulting in marked clinical improvement in motor symptoms.

Case report

A 31-year-old Brazilian woman presented with a 7-year history of fluctuating weakness and fatigue involving the lower limbs and trunk, which progressed to dysphagia, dysphonia, diplopia, and bilateral eyelid ptosis after 3 years. In the diagnostic work-up, serologic testing was positive for anti-acetylcholine receptor antibodies, and increased thymus size was found in chest CT imaging, which was compatible with thymic hyperplasia. The patient has had 7 myasthenic crises since her diagnosis and was treated with intravenous immunoglobulin several times during acute decompensation. She had no significant response to azathioprine, cyclosporin, or cyclophosphamide. She has been stable since last year, when monthly intravenous immunoglobulin therapy was started. Only minimal manifestations of fatigue and mild lower limb weakness persist.

Her medical history showed: (i) recurrent infections since childhood (urinary tract infection, recurrent sinusitis and pneumonia, including at least 5 lower respiratory tract infections in the last year);

(ii) CVID due to abnormal cellular immune response and hypogammaglobulinemia (reduced IgG, IgA and IgM serum levels); (iii) autoimmune polyglandular syndrome type 2 (type 1 diabetes mellitus, anti-GAD antibody-positive, primary adrenal insufficiency, and hypothyroidism) at 25 years of age; (iv) celiac disease at 24 years of age; (v) dermatitis herpetiformis (Dühring-Brocq disease) at 23 years of age; (vi) cerebral venous sinus thrombosis at 29 years of age; (vii) allergies to numerous foods and drugs since childhood, including latex-fruit syndrome at 18 years of age; (viii) asthma since childhood; and (ix) intestinal lymphangiectasia at 27 years of age. In the last three months, she reported subacute diarrhea, flushing, wheezing, and diffuse erythematous plaques lasting 40 days, leading to assessment for carcinoid syndrome. Positron emission tomography revealed a peripancreatic duodenal nodular lesion compatible with neuroendocrine tumor. The family history included 4 paternal aunts with malignant breast cancer, a paternal cousin with early-onset malignant breast cancer at 35 years of age, a paternal cousin with lymphoproliferative disorder at 32 years of age, and a paternal grandmother with multiple allergies. Laboratory examinations revealed variable immunological parameters, including hypogammaglobulinemia (with a mild-to-moderate reduction in serum IgG, IgA, and IgM levels), normal to mildly reduced TCD4⁺ cells and normal to mildly reduced B cells. Anemia and thrombocytopenia were not observed.

Due to her history of recurrent infection, multiple autoimmunities, and predisposition to malignancy, we performed next-generation sequencing-based multigene testing for autoinflammatory syndromes, primary immunodeficiency, and inborn errors of immunity, which revealed the heterozygous variant c.4367T>C (p.Leu1456Ser) in the *LRBA* gene (NM_001364905.1), fulfilling PM2, PP3 and PP4 criteria according to American College of Medical Genetics and Genomics guidelines (2015). This variant is very rare in the gnomAD database (AF 0.0008% in gnomAD aggregated) and is not included in the ClinVar and ABraOM databases (Online Archive of Brazilian Mutations). Multiple *in silico* prediction tools indicate that this variant has a deleterious impact (MutationTaster, Revel, Varsity, SIFT, BayesDel, fitCons, GenoCanyon). Due to the highly suggestive clinical and genomic predictions and previous reports on heterozygous variants¹³⁻¹⁶, a diagnosis of CVID8 (MIM #614700) was established.

Discussion

LRBA deficiency (or CVID8) was first described in 2012 as an autosomal recessive immunodeficiency due to biallelic homozygous or compound heterozygous variants in the *LRBA* gene (4q31.3), which codes for the LRBA protein.^{17,18} LRBA, a cytoplasmic protein that regulates intracellular vesicle trafficking and the endocytosis and exocytosis of receptors, which prevents the lysosomal degradation of cytotoxic T lymphocyte protein 4, returning it to the cell surface.¹⁹ Thus, LRBA deficiency results in reduced expression of cytotoxic T lymphocyte protein 4, an immune checkpoint protein. Affected individuals present with variable phenotypes, including childhood-onset recurrent infections (particularly sinopulmonary infections), hypogammaglobulinemia, and often multiple autoimmune disorders, in addition to lymphoproliferative and granulomatous diseases.^{5,20}

Autoimmune diseases are a common clinical feature in patients with LRBA deficiency (61%), including idiopathic thrombocytopenia purpura, autoimmune hemolytic anemia, autoimmune thyroid disease (hypothyroidism), inflammatory bowel disease, autoimmune enteropathy, type I diabetes mellitus, early-onset chronic polyarthritis, asthma, and interstitial pneumonia.²⁰⁻²² Less common manifestations include chronic atrophic gastritis, myasthenia gravis, juvenile idiopathic arthritis, anterior uveitis, autoimmune hepatitis, pancytopenia, neutropenia, allergic dermatitis, alopecia, vitiligo, urticaria and celiac disease.²¹⁻²⁴ Besides autoimmune myasthenia gravis, other neurological complications include cerebral lesions, nervous tissue atrophy, cerebral granulomas (with strabismus, hemiplegia, and seizures as complications), and granuloma-like lesions with demyelination, which result in unilateral optic nerve atrophy, opportunistic neuroinfectious diseases due to baseline immunosuppression (both humoral and cellular immune defects), and longitudinally extensive transverse myelitis.²⁵

Burkitt lymphoma, marginal zone lymphoma, lymphoproliferative diseases, and malignant gastrointestinal neoplasms have been associated with LRBA deficiency.²⁶ Patients with autoimmune myasthenia gravis are at high risk of developing thymic and extrathymic neoplasms.^{27,28} Myasthenia gravis has also been associated with carcinoid syndrome in a paraneoplastic context.^{29,30} On rare occasions, pathogenic LRBA variants have been associated with autoimmune myasthenia gravis, especially in LRBA deficiency with autoantibodies, regulatory T

cell defects, autoimmune infiltration, and enteropathy syndrome.²³ This case emphasizes the complex scenario of a monogenic mechanism leading to multiple autoimmune disorders, malignancies, and the risk of paraneoplasia.

Different medications have been used to treat LRBA deficiency, including intravenous immunoglobulin, corticosteroids, sirolimus, infliximab, rituximab, and azathioprine.³¹ More recent studies have found abatacept effective in controlling the disease, especially in immune dysregulation phenotypes, although no validated consensus exists to guide dosage, frequency, or follow-up.³² Complete or partial remission has been achieved with hematopoietic stem cell transplantation, including better immune hyperactivity control than abatacept, sirolimus, or conventional immunosuppressive therapy.¹⁹

A review of the current literature on single heterozygous variants due to possible *de novo* variants or an autosomal dominant pattern of inheritance revealed the case of a 7-year-old boy with autoimmune epilepsy, hypogammaglobulinemia, and recurrent infections.¹³ Genetic testing revealed a single heterozygous *LRBA* variant (c.6695T>C, p.Ile2232Thr), which has been previously associated with compound heterozygous variants in LRBA deficiency.¹³ Other confirmed cases of heterozygosity have been described in at least 3 clinical contexts: an adult with CVID complicated by hemophagocytic lymphohistiocytosis;¹⁴ a girl with monogenic inflammatory bowel disease who had childhood-onset chronic active colitis without granulomas;¹⁵ and an older man with juvenile-onset recurrent infections due to CVID and autoimmune thyroiditis.¹⁶

CVID with autoimmunity must be included in the differential diagnosis of patients with several associated multiple autoimmunities,^{33,34} even without a well-established family history. Autoimmune myasthenia gravis may be a neuromuscular complication in autoimmunities associated with primary immunodeficiencies, as well as in paraneoplastic syndromes. Recognizing the monogenic basis of immune dysregulation in these clinical scenarios is essential for more specific, effective, and safe therapy in neuromuscular junction disorders.

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The forbidden fruit, a case of oral allergy

O fruto proibido, um caso de alergia oral

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ABSTRACT

In adolescents and adults, 30% to 60% of food allergies are associated with pollen allergy and are included in the pollen-food syndrome (PFS). This syndrome is characterized by allergic symptoms elicited by the ingestion of fresh fruits or vegetables in patients with seasonal allergic rhinitis/rhinoconjunctivitis. The authors present the clinical case of an adolescent who, after primary sensitization to grass and olive tree pollens, subsequently manifested by cross-reactivity symptoms of oral allergy with the ingestion of fresh fruit. After diagnostic workup with the Immuno-Solid-phase Allergen Chip (ISAC) assay, profilins were identified as the proteins responsible for the cross-reactivity.

Keywords: Syndrome, pollen, fruits, adolescent, profilin.

RESUMO

Trinta a 60% das alergias alimentares em adolescentes e adultos são associadas à alergia ao pólen e estão incluídas na síndrome pólen-frutas (SPF). Esta síndrome é caracterizada por sintomas alérgicos provocados pela ingestão de frutas ou vegetais frescos em pacientes com rinite/rinoconjuntivite alérgica sazonal. Os autores apresentam o caso clínico de um adolescente que após sensibilização primária através de pólenes de gramíneas e oliveira manifestou posteriormente, por reatividade cruzada, sintomas de alergia oral com a ingestão de frutas frescas. Após recurso ao método de diagnóstico *Immuno-Solid-Phase Allergen Chip* (ISAC) verificou-se que as profilinas foram as proteínas responsáveis pela reatividade cruzada.

Descritores: Síndrome, pólen, frutas, adolescente, profilina.

Introduction

Allergic rhinitis (AR) is common in Brazil, with a mean prevalence of 29.6% among adolescents and 25.7% among children according to the International Study on Asthma and Allergies in Childhood.¹ Another study demonstrated that the incidence of seasonal allergic rhinitis (SAR) is 5% in children aged 4 years, 8.5% in children aged 6-7 years, and 19% in preadolescents.²

Thirty to 60% of food allergies in adolescents and adults are associated with pollen allergy and are included in the pollen-food allergy syndrome (PFAS).³ PFAS prevalence is significantly higher in Northern Europe because of birch pollen allergy. Osterballe et

al.⁴ estimated that 40% to 50% of patients with birch allergy had PFAS.³

PFAS is characterized by allergic symptoms that appear after the ingestion of fresh fruits or vegetables in patients with SAR/rhinoconjunctivitis.⁵ It is more common in adolescents and young adults and is more frequently characterized by the appearance of symptoms in the oral cavity after immediate contact with the food, including oral pruritus, angioedema of the lips, tongue, palate or oropharynx, laryngeal tightness, and paresthesia. This group of typical symptoms has been named oral allergy syndrome (OAS).³ However, in more severe cases (3%), there

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may be systemic involvement with gastrointestinal, cardiovascular, and respiratory tract symptoms. Thus, the terms PFAS and OAS should not be used interchangeably.⁶

This phenomenon occurs when pollen proteins cross-react with homologous food proteins. That is, proteins found in pollens and fruits and vegetables have a similar amino acid sequence that can promote specific IgE cross-reactivity, causing allergic symptoms. These proteins are called panallergens. Pathogenesis-related protein type 10 (PR-10), nonspecific lipid transfer proteins (LTPs), and profilins are the main panallergens responsible for PFAS and the best characterized to date.^{3,7} The type of protein involved in PFAS is one of the factors that can influence symptom severity.³

The estimated prevalence of PFAS in patients with pollen allergy is 47% to 70%.⁸ Clinical history is the cornerstone of PFAS diagnosis, which can be challenging, as it is not always easy to differentiate between primary sensitization, cross-reactivity, and co-sensitization.⁹ The molecules that cause PFAS are usually labile and degraded by heat and digestive enzymes.¹⁰

Profilins are proteins that cause sensitization to tree and grass pollen and are mostly found in fruits and vegetables of the *Rosaceae* family, such as apple, peach, pear, banana, mango, tomato, carrot, among others.¹¹ Patients sensitized to this group of proteins typically have mild reactions, such as OAS, and can tolerate the fruits if they are cooked.¹⁰

Clinical case

We present the case of a 14-year-old boy diagnosed with AR to grass and olive tree pollen at age 9 by an allergist/immunologist, who later developed PFAS. The patient initially reported mild oral itching after eating apples and pears, with worsening symptoms over the years, namely angioedema of the lips and tongue after eating bananas, watermelons, melons, and peaches. He denied any association with cofactors such as taking antibiotics, nonsteroidal anti-inflammatory drugs, proton pump inhibitors, physical exercise, alcohol consumption, or others. He also denied experiencing symptoms when eating other foods and had no relevant family history of allergic diseases.

Skin prick tests were performed with histamine 7 mm and saline solution 0 mm and were positive for

apple 7 mm, pear 6 mm, banana 5 mm, melon 4.5 mm, and peach 6 mm extracts. The prick by prick test was positive for apple, banana, melon, and pear and peach peel and pulp extracts.

Because the patient was polysensitized and was under multiple dietary restrictions, an ImmunoCAP™ ISAC assay was performed to elucidate the sensitization profile and characterize the patient's primary sensitization. The ISAC assay revealed high sensitivity to grass pollen due to species-specific allergens (Phl p 1, p 2, p 5, p 6, p 11) and high positivity for cross-reactive components, namely profilins (Phl p 12, Bet v 2, Hev b 8, Mer a 1). Because the patient was only sensitized to profilins, an oral food challenge with cooked apple was conducted, which was negative.

The patient was prescribed oral antihistamines and intranasal corticosteroids for use during the pollen season. In case of accidental ingestion of any of the previously mentioned foods, the patient was also prescribed emergency drugs including antihistamines, systemic corticosteroids, and an epinephrine auto-injector for anaphylaxis. He was advised to avoid eating pears, bananas, watermelons, peaches, and melons.

Conclusion

In this clinical case, primary sensitization occurred via the respiratory route through grass and olive tree pollen. The patient later developed OAS to fresh fruit as a result of cross-reactivity due to sensitization to profilins. Since these proteins are unstable when digested by pepsin and are degraded by heat, we expected the patient to tolerate cooked fruit, as shown in the oral food challenge with cooked apple.¹⁰

Performing the ISAC assay was crucial for ruling out the involvement of proteins such as PR-10 and LTPs, which are associated with more severe reactions. This allowed us to describe the profile of the polysensitized patient and achieve a possible prognosis of reaction severity.

In clinical practice, if a patient has sensitization to grass and/or tree pollen, it should always be investigated whether there is a history of symptoms related to fruit or vegetable ingestion. Patients should be informed about the characteristics and distribution of the allergens to which they are sensitized.

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Proficiency testing for the skin prick test: the pressing need for quality certification among ASBAI specialists

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Skin prick tests (SPTs) are difficult to standardize, and SPT performance depends primarily on the experience of the tester. The SPT is an important diagnostic procedure in clinical allergy, but quality documentation is often not performed. The effect of several factors, such as type of device, shape, material, and applied force, has not yet been extensively investigated.

With the goal of investigating the effect of these factors on SPT performance, Chiirananirungroj et al. evaluated 4 devices with different shapes and materials that were applied on 12 subjects under 3 different forces (30, 45, and 60 g). The results were compared with the standard method using an ALK lancet pricked by an experienced clinician. A total of 480 pricks were performed on 12 patients. Papule size and sensitivity increased with higher applied forces in all devices. Thinner lancets with a long, sharp tip had relatively higher analytical sensitivities and provided 100% sensitivity at applied forces ≥ 45 g. The pain scores for all devices at applied forces from 30 to 60 g ranged from 1.00 to 1.81, with minimal bleeding incidences (0%-4.17%). The pain score for the standard method with the ALK lancet was 2.08, with a much higher bleeding incidence (27.1%). Device type and shape and applied force are the key factors affecting SPT performance. These results could pave the way for improved SPT performance and standardization.¹

A study conducted in Curitiba aimed to compare the results obtained with needle vs. Multi-Test II® in SPTs with different concentrations of histamine and *Dermatophagoides pteronyssinus*, as well as the pain reported by patients with each device. The study included 104 children aged from 6 to 15 years with a diagnosis of asthma and/or rhinitis and/or atopic dermatitis and with

a positive SPT for *Dermatophagoides pteronyssinus*. The tests were performed using 13 x 0.3 BD Precision Glide® disposable hypodermic needles and Multi Test II® with histamine 10 mg/mL and 1 mg/mL, *Dermatophagoides pteronyssinus* extract 5,000 PNU/mL and 10,000 PNU/mL, and saline solution. Pain was assessed after each test using the Wong-Baker Faces Pain Rating Scale. SPT sensitivity was 100% for the two devices with histamine 10 mg/mL. With histamine 1 mg/mL, the Multi-test II® showed higher sensitivity ($S = 86.5\%$) than the needle ($S = 56.7\%$). A high degree of agreement was observed between both devices with *Dermatophagoides pteronyssinus* extract at the 10,000 PNU/mL concentration; with 5,000 PNU/mL, the degree of agreement was 69.1% ($\kappa = 0.2$). Pain was reported by 65 children (62.5%) with the Multi-Test II® and by 48 (46.2%) with the needle ($p = 0.01$). Both devices showed high sensitivity. There were differences in papule size between the devices, but false-positive results were rarely observed.²

A proficiency system has been described to evaluate staff members in relation to the internationally recommended reproducibility in terms of coefficient of variation ($CV < 40\%$) and linearity (coefficient of regression > 0.85) based on blinded histamine testing using histamine 3, 10, 30, and 100 mg/mL. Fourteen allergy nurses participated in the proficiency test. More than 95% of nurses had a CV below 40%, and approximately 35% had a CV below 20% based on papule area. As for linearity (coefficient of regression), only 2 nurses produced tests with a value lower than 0.85, and 79% of nurses had a coefficient of regression higher than 0.95. Depending on the gentleness of the SPT, variability between nurses in the papule area varied more than twofold, corresponding to a 10-fold increase in histamine concentration. This would never have been detected without the use of a proficiency testing system. The described histamine test provides an objective system to evaluate basic SPT quality and assessment standards, especially for documentation in scientific studies.

In view of the lack of standardization for SPT, the divergent results between devices and testers, the lack of documentation of standardized assessments and reproducibility of results among residents and graduate students during training, and the fact that performing and interpreting SPTs in Brazil is considered a medical

procedure, there is an urgent need for ASBAI to conduct SPT proficiency testing to safeguard the health of patients with allergies and guarantee the quality of the service provided.

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