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Near fatal asthma: treatment and prevention

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Key words
near fatal asthma; acute asthma; severe asthma; asthma attack; asthma exacerbations; asthma-related deaths

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Summary
Near-fatal asthma (NFA) is described as acute asthma associated with a respiratory arrest or arterial carbon dioxide tension greater than 50 mmHg, with or without altered consciousness, requiring mechanical ventilation. Risk factors for near fatal asthma have not been fully elucidated. In 80-85% of all fatal events, a phenotype, characterized by eosinophilic inflammation associated with gradual deterioration occurring in patients with severe and poorly controlled asthma, has been identified. Regarding to the management, acute severe asthma remains a significant clinical problem, which needs to be identified to facilitate early and appropriate therapeutic interventions. The assessment relies on clinical signs, but additional information might be obtained from chest radiography or blood gas analysis. No investigation should delay the initiation of appropriate therapy. The goals of therapy are the maintenance of oxygenation, relief of airflow obstruction, reduction of airways edema and mucus plugging (with Increased use of medications such as beta-agonists via metered dose inhalers and nebulizers, oral and/or intravenous (other than by inhalation) corticosteroids and oral or intravenous theophylline) whereas supporting ventilation as clinically indicated. Of course, the emergency physician needs to consider the wide range of potential complications, as attention to these problems when managing severe acute asthma might significantly improve outcome. An understanding of the available agents and potential pitfalls in the management of NFA is mandatory for the emergency physician.

Background

Asthma is a significant public health problem that is increasing in prevalence and is associated with relevant morbidity and financial costs (1,2). There is suggestion that asthma-related deaths are decreasing, but a significant minority of individuals presents with severe asthma and have persisting daily symptoms, and exacerbations despite compliance with high doses of inhaled steroids and additional treatment. For this small part of the asthmatic population, the exacerbation can become fatal or near-fatal (1-10). These observations appear to be paradoxi-

cal with the increasing knowledge of asthma pathogenesis and treatment that is currently available. Near-fatal asthma (NFA) is described as acute asthma associated with a respiratory arrest or arterial carbon dioxide tension greater than 50 mmHg, with or without altered consciousness, requiring mechanical ventilation (8). Two distinctive phenotypes of NFA have been identified. The most common phenotype, responsible for 80-85% of all fatal events, is characterized by eosinophilic inflammation associated with gradual deterioration over days or weeks occurring in patients with severe and poorly controlled asthma, and is slow to respond to therapy. This phenotypic pattern is generally considered preventable. The second phenotype, with neutrophilic inflammation, has both rapid onset and response to therapy (4,7,11).

Risk factors

Remodeling in asthma refers to structural changes in large and small airways, consisting of subepithelial fibrosis, increased vascularity, increased airway smooth muscle mass, and goblet cell hyperplasia of proximal and distal airways. Remodeling was believed originally to be the cause of refractory asthma, that is, asthma that fails to respond to optimal treatment and is characterized by persistent airflow limitation. A history of intensive care admission or mechanical ventilation is a well-documented indicator of subsequent NFA (12). Gelb et al. found that in NFA patients the sensitivity for the presence of moderate and/or severe obstruction was 90%, the specificity was 61%, the positive predictive value was 41%, and the negative predictive value was 95%. The sensitivity for an abnormal loss of lung elastic recoil (i.e., less than the predicted normal mean _1.64 SD) was 100%, the specificity was 79%, the positive predictive value was 59%, and the negative predictive value 100% for NFA patients (13). Using TLC percent predicted as a surrogate for elastic recoil, the sensitivity for TLC of > 115% predicted was 70%, the specificity was 70%, the positive predictive value was 88%, and the negative predictive value was 41% for NFA patients (13). Using the ratio of FEV1 percent predicted to TLC percent predicted of < 0.70, the sensitivity was 90%, the specificity was 78%, the positive predictive value was 56%, and the negative predictive value was 96% for NFA patients (13). The unexpected loss of lung elastic recoil in patients with chronic persistent asthma, and its significant physiologic contribution to adverse clinical complications including NFA, are novel prospective observations. This loss of lung elastic recoil was associated with increasing age, duration of asthma, and severity of expiratory airflow limitation, using postbronchodilator FEV1 percent predicted as the signal. Additionally, normal transdiaphragmatic pressures, despite the presence of hyperinflation in patients with NFA, extend similar observations about asthmatic patients without NFA (6,14). Postmortem series show pathological presence of inflammatory cells, mucus plugging, shedding of airway epithelium, airway oedema and smooth muscle hypertrophy (6,14). Airway obstruction in severe asthma that does not respond to conventional therapy, may be caused by mucus plugging (6,14). Evidence for the management of mucus plugging in adult patients with severe near fatal asthma is sparse. Chia et al. describe a patient with fatal asthma who responded dramatically to DNase following bronchoscopy and lavage after failing other therapies in a case report, and believe that the combined use of rhDNase, bronchial toileting and aggressive physiotherapy, on top of mechanical ventilation strategies and intravenous bronchodilators, helped turn the corner (15).
More recently Serrano-Pariente et al. analysed 179 asthmatics patients admitted to the hospital for an episode of NFA. Three clusters of patients with NFA were identified: cluster 1, the largest, including older patients with clinical and therapeutic criteria of severe asthma; cluster 2, with an high proportion of respiratory arrest, impaired consciousness level and mechanical ventilation; and cluster 3, which included younger patients, characterized by an insufficient anti-inflammatory treatment and frequent sensitization to Alternaria alternata and soybean (7).

Assessment

In a study of asthma patients admitted with a near-fatal episode, two-thirds of subsequent severe attacks or deaths occurred within 1 year of the previous life-threatening admission (16). The immediate assessment of patients with asthma should include the degree of respiratory distress (ability to speak, respiratory rate, use of accessory muscles, air entry), degree of hypoxia (cyanosis, pulse oximetry, level of consciousness) and cardiovascular stability (arrhythmias, blood pressure). Accessory muscle use, wheeze and tachypnoea might diminish as the patient tires (17) **(table 1)**. The clinical examination might be misleading; occasionally asthmatics with poor perception of the severity of their asthma appear deceptively well, despite severe decrements in lung function. Although the assessment relies on clinical signs, additional information might be obtained from chest radiography or blood gas analysis. No investigation should delay the initiation of appropriate therapy. On chest radiography, an episode of acute asthma is characterized by hyperinflation of the lungs. Physiologically abnormal distribution of ventilation, perfusion and altered gas exchange. Expiratory flow limitation with incomplete expiration leads to hyperinflation of the lungs, adding to the elastic burden of the thorax. Passive elastic recoil is no longer sufficient to achieve effective expiration, and expiratory muscles are then actively involved in expiration (4,12). Progression of dynamic hyperinflation is associated with a higher intrathoracic pressure at the end of expiration (intrinsic Positive End Expiratory Pressure - iPEEP or auto-PEEP). Hyperinflation and higher intrathoracic pressures mean the respiratory muscles

Table 1 *- Markers of severe asthma.*

Inability to speak in full sentences
FEV1 < 40%, predicted or PEF < 40% of best or predicted (< 25% in life-threatening asthma)
Oxygen saturations < 90-92%
PaO2 < 60 mmHg - PaCO2 > 45 mmHg
Use of accessory muscles or tracheal tugging
Pulsus paradoxus (> 15 mmHg decrease with inspiration). With severe muscle fatigue might be absent
Quiet chest on auscultation
Patient seated upright and unable to lie supine
Cyanosis and sweating
Confusion or decreased level of consciousness - Hypotension or bradicardia

Table 2 *- Asthma complications.*

Pneumothorax
Pneumomediastinum - Pneumopericardium
Pulmonary interstitial emphysema
Pneumoretroperitoneum
Cardiac arrhythmias
Myocardial ischaemia or infarction
Mucus plugging - Atelectasis - Pneumonia
Electrolyte disturbances (hypokalaemia, hypomagnesaemia, hypophosphataemia)
Lactic acidosis - Hyperglycaemia
Theophylline toxicity

start at greater stretch (hence less efficient, more fatiguable), and greater inspiratory effort is needed to commence flow into the lungs, which also increases work of breathing (4,12). Barotrauma, which refers to the adverse effects of this increased intra-lung pressure on both the lung structure as well as that transmitted to the vascular structures, can result in bullae rupture, pneumothorax and reduced venous return with hypotension. Blood gas analyses might reveal respiratory alkalosis, hypoxaemia and hypocarbia. Generally, asthma attacks are not characterized by marked arterial desaturations until very late in life-threatening episodes. Hypercarbia occurs in 10% of cases presenting to the ED (4,12). These patients have greater airway obstruction and respiratory rate than non-hypercapnic patients. A quiet chest on auscultation, inability to talk and cyanosis suggest the presence of hypercarbia. The finding of normocarbia in acute asthma should also be viewed as a sign of impending respiratory failure that requires aggressive treatment, Patients who fail to respond to therapy (PEFR improved by less than 10-20%) or with persistent hypercapnia, tachypnoea (respiratory rate _30), altered mental status, arrhythmias or significant comorbidities should be referred to the ICU (4). The emergency physician also needs to consider the wide range of potential complications, as attention to these problems when managing severe acute asthma might significantly improve outcome **(table 2).**

Therapy

The goals of therapy are the maintenance of oxygenation, relief of airflow obstruction, reduction of airway o edema and mucus plugging whereas supporting ventilation as clinically indicated. High-flow oxygen has been assumed to be harmless, and is often used in the treatment of patients with acute asthma (18). Hypoxaemia in asthma results from ventilation / perfusion mismatching and is thus usually easily corrected with modest increases in the fraction of inspired oxygen (e.g. 1-3 L/min via a nasal cannula or mask). Uncontrolled oxygen has been postulated to correct the effects of hypoxaemia and to compensate for any trend towards a fall in arterial oxygen tension associated with b2-agonist therapy (19). Short-acting, inhaled b2-agonists are the drugs of choice for treating acute asthma. Their onset of action is rapid and their side-effects are well tolerated. Salbutamol, the most frequently used b2-agonist in ED around the world, has an onset of action of 5 min and a duration of action of 6 h. b2-agonists have been described as rescue therapy for use in patients unresponsive to inhaled bronchodilator and systemic corticosteroid therapy, or when the inhaled route is not practical (20). The safety profile of short-acting-β2-agonists has been questioned due to possible detrimental effects on asthma control. Recent evidence and meta-analysis suggest an increased risk for cardiovascular complications in patients using β2-agonists (21,22). There is evidence suggesting that the frequent use of these drugs might increase the risk of premature death. The hypothesis that β2-agonists can have fatal adverse effects was first demonstrated in the late 1960, when Inman and Adelstein reported a 30-700% increase, depending on age, in asthma death in patients using pressurized aerosol containing, most often, isoprenaline (23). The excess mortality was attributed to overdosing but β-agonists came into focus again in the 1980, when more selective β2-agonists were introduced in metered-dose inhaler. A dose-dependent risk of death from asthma was reported, with increased up to 29-fold with the use of β2 agonists (24). Afterwards, large, randomised, double-blind

trials have been performed to test the hypothesis that the use of LABAs in asthmatic patients is associated with an increased risk of death (25). At present, patients with asthma should be initiated and maintained on sufficiently high doses of inhaled corticosteroids and only patients whose asthma cannot be controlled should receive additional β2-agonists on a regular basis in addition, LABA should be withdrawn from patients who do not profit from their use (1). The need for reliever medication, such as the inhaled short-acting β2-agonists (SABA) albuterol, along with daytime symptoms, nighttime waking and activity limitations, is used to assess symptom control in asthma and to estimate the risk of future exacerbations (1).

The inclusion of reliever inhaler use in assessment of asthma control in adults, used on evidence that overuse of SABA medication is associated with poor symptom control, increased risk of exacerbations and death from asthma (26).

Adrenaline has been used both as a nebulized solution and intravenously. There are theoretical advantages to the preferential use of i.v. adrenaline as opposed to pure b2-agonists in acute severe asthma. Although bronchoconstriction is the major pathology in asthma, airway oedema might also make a significant contribution. Both the a-agonist and β-agonist effects of adrenaline might be beneficial, with the a-effect decreasing oedema and the b-effect responsible for bronchodilation. Anticholinergics agents block muscarinic receptors in airway smooth muscles, inhibit vagal cholinergic tone and result in bronchodilation. Ipratropium bromide has a mild additional bronchodilating effect when added to b-agonists, that might only be significant in severe asthma (20). Because anticholinergic agents and b2-agonists exert effects by different mechanisms, affect different-sized airways and have different pharmacodynamic and pharmacokinetic properties, the combined use of them is rational and is likely to result in improved bronchodilation. Corticosteroids have been shown to improve asthma symptoms by reducing airway inflammation, airway reactivity and decrease airway secretions. In addition to their anti-inflammatory effect, steroids increase the number and sensitivity of b-receptors on the bronchial smooth muscle (28,29). Objective improvements in airflow obstruction have usually not been demonstrated during the first 6-12 h of treatment with corticosteroids in acute asthma (30). Corticosteroids are recommended for most patients in the ED, particularly in those who do not respond completely to initial b2-agonist therapy. Corticosteroid administration reduces admission rates, decreases relapse rates and might also reduce the number of cases of fatal asthma. Because benefits from corticosteroid treatment are not usually seen for 6-24 h after administration, therapy should be instituted early. Low dose corticosteroids (_80 mg/day of methylprednisolone or _400 mg/day of hydrocortisone) appear to be adequate in the initial management of adult patients (31). Higher steroid doses do not appear to offer a therapeutic advantage, and because the risk of myopathy is significant, especially in the mechanically ventilated patients, the concomitant use of systemic corticosteroids and paralytic agents should be avoided if at all possible. Importantly oral and i.v. routes of corticosteroid administration are equally efficacious with respect to rate of resolution of airflow limitation (32). The parenteral route is required in patients unable to take oral medication (intubated) or if absorption might be compromised (e.g. vomiting). There is some suggestion that for patients with severe symptoms, i.v. corticosteroid therapy might have an early effect (within 1-6 h) by reversing b2-receptor downregulation seen in chronic b2-agonist use (33). The use of i.v. aminophylline was associated with a higher incidence of adverse effects compared with standard care alone (34). Whether aminophylline has a place as an additional therapy after treatment with established medications such as inhaled b-agonists, systemic corticosteroids and i.v. magnesium remains uncertain. At the current time routine use of aminophylline in severe asthma cannot be recommended. Magnesium might be effective in acute asthma through a variety of mechanisms. This cation is an important cofactor in many intracellular enzymatic reactions. Magnesium has been shown to relax smooth muscle and might be involved with inhibition of smooth-muscle contraction. As an explanation for the effects of magnesium in acute asthma this is perhaps overly simplistic. Magnesium is involved in acetylcholine and histamine release from cholinergic nerve terminals and mast cells, respectively. Furthermore, the ability of magnesium to block the calcium-ion influx into the bronchial smooth muscle might have therapeutic benefit in severe acute asthma (35). A single dose of i.v. MgSO4 administered to patients with severe acute asthma has been shown to be effective. A multicentre trial demonstrated that 2 g of i.v. magnesium sulphate administered as an adjunct to standard therapy, improved pulmonary function in patients presenting to the ED with severe asthma. i.v. (36). Montelukast in addition to standard therapy produces rapid benefit and is well tolerated in adults who have acute asthma. Patients with severe bronchospasm requiring mechanical ventilation and not responding to conventional bronchodilator therapy might benefit from an inhaled volatile anaesthetic agent with bronchodilating properties such as halothane, enflurane or isoflurane (37-41). Use of these agents might result in hypotension and cardiac dysrhythmias, especially in hypoxic patients. Administration is complex and requires either an anaesthetic machine or alternative heat moisture connector device. For practical reasons this therapy is better reserved for use in the ICU. Despite appropriate therapy, there continues to be a small group of patients who deteriorate or those who present in extremis and require mechanical ventilation. The rate of intubation in patients with acute severe asthma is low at 3-8% (42). Surprisingly, only a few reports have described the use of

non-invasive ventilation (NIV) in patients with acute severe asthma (43,44). The positive pressures employed in the studies to date are generally less than 15 cm H_2O, and whether CPAP or BiPAP is the optimal approach remains unknown. Although there are some similarities between asthma and chronic obstructive pulmonary disease, in asthma CO_2 retention occurs late in the exacerbation and by that time the patient is often exhausted and has difficulty tolerating the NIV mask. Furthermore, patients with severe acute asthma are usually tachypnoeic and might struggle to coordinate their breathing with that of the machine and therefore find BiPAP uncomfortable. Mucous production is a feature of severe acute asthma and NIV can exacerbate sputum retention, and it is important that this is borne in mind when implementing NIV. A Cochrane review performed in 2005 concluded there are promising results in favour of the use of NPPV in severe acute asthma; however, the regular use of NPPV in status asthmaticus still remains controversial. Until large randomized controlled trials are completed, this therapy should be restricted, and routine clinical use cannot be recommended (45).

Invasive ventilation

Deteriorating consciousness, severe exhaustion and cardiopulmonary arrest are absolute indications for intubation and mechanical ventilation. Severe hypercapnia, acidosis and fatigue might not warrant immediate intubation, but rather aggressive and continuous bronchodilator therapy. Intubation and mechanical ventilation in the asthmatic should not be embarked upon lightly. Once it is apparent that invasive ventilation is required, experienced help should be sought. The optimal means of intubation is usually direct laryngoscopy, following rapid sequence induction. The best agents to use are those most familiar to the operator. Induction might effectively be achieved with propofol or thiopentone; however careful dosage adjustment is required for potential haemodynamic compromise. The asthmatic patient is often volume-depleted, with induction resulting in both loss of sympathomimetic tone and drug-induced vasodilation. Additional to this, the development of intrinsic PEEP with an inappropriate ventilation strategy might rapidly result in catastrophic circulatory collapse. In this regard ketamine with its sympathomimetic and bronchodilating properties has been advocated by many as the induction agent of choice (46). Inhalational volatile induction is attractive given the bronchodilating properties of these agents, and it might obviate the need for paralysis. It however requires specialized anaesthetic skills and equipment to be available in the emergency room, as transfer of such critically ill patients would be ill advised. Following induction, maintenance with fentanyl and midazolam is appropriate. Fentanyl is the opiate of choice because it inhibits airway reflexes, causes less histamine release than morphine, but on rare occasions can induce chest wall rigidity with rapid bolus dosing (47). Ongoing paralysis might initially be required to facilitate ventilation; however, because of the significant risk of critical illness polyneuromyopathy (especially given the combination with steroids), neuromuscular blockade should be withdrawn as soon as possible. The incidence of myopathy in asthmatics on long-term nondepolarizing neuromuscular blocking agents has been reported as high as 30% (48). The mode of ventilation might be a crucial factor for a successful outcome of NFA. Mechanical ventilation is often difficult because the obstructive defect might result in dynamic hyperinflation. This might then lead to barotrauma, volutrauma or catastrophic haemodynamic compromise secondary to impairment of venous return. Regardless of the mode of ventilation selected, the goals of mechanical ventilation are to maintain adequate oxygenation, minimize dynamic hyperinflation, avoid barotrauma and accept some degree of hypercapnia until bronchodilators and steroids improve airflow. Outcome is improved in mechanically ventilated asthmatics by limiting airway pressure using a low respiratory rate and tidal volume, whereas permitting a moderate degree of hypercarbia and respiratory acidosis (49). Hypercarbia has not been found to be detrimental, except in patients with severe myocardial depression. Moderate degrees of hypercarbia with an associated acidosis (pH 7.15-7.2) are generally well tolerated. To prolong the expiratory time and allow adequate time for expiration, the breath rate can be reduced, or inspiratory time decreased thereby extending the inspiratory to expiratory (I:E) ratio to much greater than 1:2. Expiration should ideally be observed both clinically and on the ventilator graphics to be complete before the next breath is delivered. Pressure control ventilation might not be an ideal mode of ventilation for patients with NFA, as frequent fluctuations in airway resistance lead to variable tidal volumes and a risk of significant hypoventilation. The use of extrinsic PEEP remains controversial, in mechanically ventilated paralysed patients, and Tuxen found it to be of no benefit at low levels and detrimental at high levels, because the decrease in gas trapping was replaced by a rise in functional residual capacity (50). It should be noted that very large tidal volumes were used in the present study. Extrinsic PEEP might prevent airway collapse by splinting the airways open (51); however, as a general rule, extrinsic PEEP should not exceed intrinsic PEEP, and ongoing clinical assessment for the presence of gas trapping and magnitude of FRC are mandatory. Ensuring adequate humidification of inspired gas is particularly important in the ventilated asthmatic, to prevent further thickening of secretions and drying of airway mucosa that might stimulate further bronchospasm. Finally, it should be noted that mechanical ventilation might compromise delivery of aerosolized bronchodilators. Drug delivery might vary from 0% to 42% in ventilated patients, and it is therefore important

to ensure compatibility between the delivery system and ventilator circuit used (52).

Table 3 *- Initial ventilator settings in paralysed asthmatic patients.*

FiO2 1.0, then titrate to keep SpO2 > 94%
Tidal volume 5-6 mL/kg
Ventilator rate 6-8 breaths/min
Long expiratory time (I:E ratio > 1:2)
Minimal PEEP _5 cmH2O
Limit peak inspiratory pressure to < 40 cmH2O
Target plateau pressure < 20 cmH2O
Ensure effective humidification

Conclusion

Acute severe asthma remains a significant clinical problem, which needs to be identified to facilitate early and appropriate therapeutic interventions. In this regard the identification of patients with a specific NFA phenotype could be helpful to prevent future severe asthma exacerbations. An understanding of the available agents and potential pitfalls in the management of NFA is mandatory for the emergency physician.

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Food allergy in breastfeeding babies. Hidden allergens in human milk

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Summary
Background. *Food allergy is a rare disorder among breastfeeding babies.* ***Objective.*** *Our aim was to identify responsible allergens in human milk.* ***Methods.*** *We studied babies developing allergic symptoms at the time they were breastfeeding. Skin prick tests (SPT) were performed with breast milk and food allergens. Specific IgE was assessed and IgE Immunoblotting experiments with breast milk were carried out to identify food allergens. Clinical evolution was evaluated after a maternal free diet.* ***Results.*** *Five babies had confirmed breast milk allergy. Peanut, white egg and/or cow's milk were demonstrated as the hidden responsible allergens. No baby returned to develop symptoms once mother started a free diet. Three of these babies showed tolerance to other food allergens identified in human milk.* ***Conclusion.*** *A maternal free diet should be recommended only if food allergy is confirmed in breastfed babies.*

Introduction

Breast milk is the optimal nutrition for infants. Whether breastfeeding protects against the development of allergies remains controversial (1,2,3). Some studies report protection with exclusive and prolonged breastfeeding (4,5,6), particularly in children prone to atopy (7,8). Other reports have suggested breast milk could be responsible for early sensitization to food (9,10,11). In a high-risk cohort, McGowan et al. (3) found an extremely high cumulative incidence of food allergy associated with breastfeeding. Food proteins ingested by women who are breastfeeding are absorbed and excreted into breast milk antigenically active. Eczema, colic, diarrhea and vomiting are frequent symptoms in exclusively breastfed infants, but rarely food allergy has been demonstrated in this group (12,13). In a multidisciplinary review of the literature concerning the impact of early feeding in infancy on later allergic manifestations, Van Odijk et al. (14) concluded that breastfeeding protects against the development of atopic disease, and this effect appears even stronger in children with atopic heredity.

We studied babies developing allergic symptoms at the time they were breastfeeding. The aim of our study was to identify breast milk allergens involved in allergic reactions after breastfeeding.

Methods

Infants with immediate erythema, hives, vomiting, diarrhea, sneezing, coughing or breathlessness, during or within 1 hour of breastfeeding, were included in this study. The study was carried out in accordance with the ethical standards established in the Declaration of Helsinki. A written informed consent document, previously approved by the Ethics Committee (Hospital La Paz) was provided by the mothers before beginning the study and collecting the breast milk samples. The mothers provided us with

information about foods tolerated by the infant and a detailed description of the foods ingested by themselves before breastfeeding, and the symptoms developed by their baby. We studied food sensitization in the infants and looked for hidden allergens in the breast milk identifying the responsible food allergens there.

Breast milk samples (≥ 30 ml) were collected from the babies' mothers: (A) samples collected 24 hours after following a diet free of any suspected food, including those for which the infant had a positive skin prick test (SPT) or specific IgE (sIgE); and (B) samples collected at 2-hour intervals up to 8 h post-dietary challenge with each one of the suspected implicated foods (250 ml cow's milk, 1 hen's egg, 100 g of hake or 30 g of peanuts). A 24 h period free from ingestion of any suspected food was required before ingesting any other food and subsequently collecting the corresponding samples. Breast milk samples were stored at -20°C after collection. Milk was defatted by centrifugation (2000 g, 20 min at + 4°C) before the different assays.

Skin tests: SPT with cow's milk, α-lactalbumin (Bos d 4), β-lactoglobulin (Bos d 5), bovine serum albumin (Bos d 6) and casein (Bos d 8); hen's egg white; hake; lentil and peanut (DIATER Laboratories, Spain) and skin prick-by-prick tests (SPPT) with corresponding collected samples of the mother's breast milk were performed on the infants. Histamine 1/100 and glycerinate saline were used as positive and negative controls. The averages of the diameters of the wheal were assessed after 15 minutes.

IgE: Serum total and specific IgE (sIgE) were determined by Immuno CAP® (Phadia).

An immunoblot analysis of immunoglobulin E and immunoblot inhibition experiments were performed for each patient's serum with the suspected foods and respective breast milk samples to identify hidden food allergens. Briefly, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing and denaturing conditions. Protein extracts from suspected foods and breast milk collected at various times were loaded onto 15% gels and stained with Coomassie blue to visualize constitutive protein bands and for Western blot analysis. The separated proteins were electro-transferred onto nitrocellulose membranes and blocked with 0.5% PBS Tween. The membranes were incubated with individual samples of diluted sera and suspected foods in the case of inhibition, and then were reacted with a mouse anti-human IgE Fc-HRP (Southern Biotech, Birmingham, USA). After additional washing, the bands were made visible on the membrane using chemiluminescent development substrates.

Food tolerance was defined as the patients eating a specific food without symptoms occurring. In the case of food sensitization without previous ingestion, tolerance was assessed upon the introduction of the food by a controlled open food challenge. The clinical evolution of the infants was evaluated after establishing an infant and maternal free diet of identified allergen.

Results

Forty-seven breastfed babies (1-19 months) referred with immediate symptoms (urticaria, erythema or vomiting) at the time they were breastfeeding were evaluated to food allergy. Only five infants who had proved allergic symptoms after breastfeeding were studied.

Case 1: A 6-month-old girl with atopic dermatitis since her first month of life and exclusively breastfed suffered, from her second month, immediate erythema and pruritus on her face during breastfeeding. At 6 months of age, she developed generalized urticaria and vomiting 10 minutes after consuming her first bottle of humanized cow's milk formula. Specific IgE to cow's milk proteins was demonstrated in her serum and she was diagnosed with cow's milk allergy. Her mother started a cow's milk-free diet and the infant was fed with mixed breastfeeding with a hydrolyzed cow's milk formula and subsequently meat, fish, egg and lentils. When the baby was 10 months old, she experienced a new episode of hives around her mouth while breastfeeding. Her mother reported that 2 hours before she had eaten peanuts.

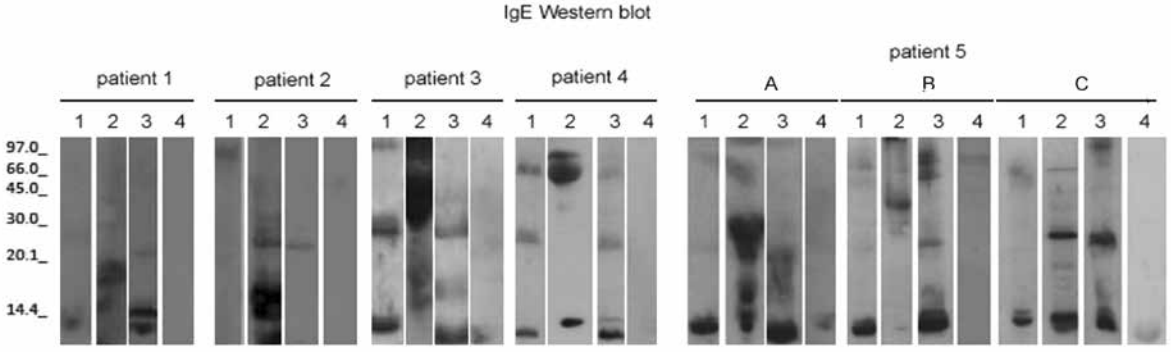
Case 2: A 14-month-old girl breastfed from birth who was tolerating cow's milk formula, meat, fruits and vegetables from eight and fish from ten months, developed hives affecting her face after ingesting a bottle of humanized cow's milk continuing breastfeeding. Her mother remembered that she had eaten peanuts 1 hour before breastfeeding. She had suffered from wheezing and atopic eczema from her second month.

Case 3: An exclusively mixed breastfeeding 3.5-month-old girl, suffering mild eczema from birth, began to develop recurrent hives on her face during breastfeeding. She had been tolerating humanized cow's milk formula since 1.5 months of her life. Her mother did not relate the appearance of symptoms with her previous ingestion of any suspicious food.

Case 4: An 8-month-old boy exclusively breastfed developed eczema on his face from 1 month of age. At 3 months of age he began to experience discomfort and occasional vomiting in the first hour after breastfeeding. He had started mixed feeding at 4 months with a humanized cow's milk formula and subsequently he had been tolerating cereals, meat and fruit. At 5 months of age, he developed uneasiness and vomiting, pruritus, erythema and worsening of his eczema 30 minutes after breastfeeding. His mother did not associate the appearance of symptoms with prior ingestion by herself of any particular food.

Case 5: A breastfed 15-month-old girl, suffering from atopic dermatitis and wheezing from her second month, developed at 5 months of age an allergic reaction (generalized urticaria, vomiting, respiratory distress and cyanosis) 10 minutes after ingesting 20 ml of a humanized cow's milk formula. Symptoms were controlled after applying adrenaline, corticosteroids and antihistamine treatment. Specific IgE to cow's milk proteins was

Figure 1 *- Recognition of food allergens by patients' sera in the breast milk samples (A collected more than 24 h after ingesting specific food/s and before ingesting the specific food; sample B collected 4-8 h after ingesting the specific food).*



Patient 1. Lane 1: breast milk simple A collected before peanut intake. Lane 2: peanut. Lane 3: breast milk sample B collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.
Patient 2. Lane 1: breast milk A sample collected before ingesting peanut. Lane 2: peanut. Lane 3: breast milk B simple collected after ingesting peanut. Lane 4: breast milk B sample collected after ingesting peanut and inhibited with peanut.
Patient 3. Lane 1: breast milk A sample collected before ingesting hen's egg. Lane 2: white egg. Lane 3: breast milk B sample collected after ingesting white egg. Lane 4: breast milk B sample collected after ingesting egg and inhibited with white egg.
Patient 4. Lane 1: breast milk A sample collected before ingesting hen's egg. Lane 2: white egg. Lane 3: breast milk B sample collected after ingesting egg. Lane 4: breast milk B simple collected after ingesting white egg and inhibited with white egg.
Patient 5. A. Lane 1: breast milk A sample collected before ingesting egg, cow's milk or peanut. Lane 2: cow's milk. Lane 3: breast milk B sample collected after ingesting cow's milk. Lane 4: breast milk B sample collected after ingesting cow's milk and inhibited with cow's milk. *B.* Lane 1: breast milk A sample collected 24 h after ingesting egg, cow's milk or peanut. Lane 2: egg white. Lane 3: breast milk B sample collected after ingesting white egg. Lane 4: breast milk B simple collected after ingesting white egg and inhibited with white egg. *C.* Lane 1: breast milk sample collected 24 h before ingesting egg, cow's milk or peanut. Lane 2: peanut. Lane 3: breast milk B simple collected after ingesting peanut. Lane 4: breast milk B simple collected after ingesting peanut and inhibited with peanut.

detected in her serum and she was diagnosed with cow's milk allergy. Thereafter, she and her mother began a diet free of cow's milk. At the age of 10 months, she developed urticaria around her mouth while she was breastfeeding. Her mother did not recall the food she had eaten before breastfeeding. The infant was tolerating cereals, vegetables, fruits, meat and fish.

Table 1 shows detailed data concerning patients (age, sex, atopic dermatitis or asthma and results of SPPT with breast milk) and the mothers' atopic diseases. **Table 2** shows data about SPT total and specific IgE and tolerance to cow's milk, hen's egg, hake and peanut. No patient was sensitized to lentils. All 5 infants were sensitized to hen' egg white, but 2 of them could tolerate it. Three were sensitized to cow's milk but only one of them (Case 2) had tolerance. None of the infants had introduced peanuts in their diet but three of them had specific IgE to peanuts. Two infants were sensitized to hake, and both of them had eaten it without any symptoms. **Figure 1** shows an image with the results of the immunoblot and immunoblot inhibition experiments.

Case 1 had a positive SPPT to breast milk sample B obtained after the mother had ingested peanuts. IgE in the patient's serum recognized 2 proteins of approximately 14 kDa in the breast milk collected after the ingestion of peanuts, and both were inhibited by peanuts. Case 2 had positive SPPT to breast milk sample B, obtained after the mother had ingested peanuts. IgE in the patient's serum recognized proteins of approximately 30 kDa in the collected breast milk after the ingestion of peanuts that were inhibited by peanuts. Case 3 had positive SPPT to breast milk sample B, obtained after the mother had ingested egg. IgE in the patient's serum recognized a group of proteins of approximately 14 and 30 kDa in the collected breast milk after the ingestion of hen's egg, and this recognition was inhibited by egg white. Case 4 had a positive SPPT to breast milk sample B, obtained after the mother had ingested hen's egg. IgE in the patient's serum recognized a group of proteins of approximately 14 and 30 kDa in the breast milk collected after the ingestion of hen's egg; this recognition was inhibited by egg white. Case 5 had a positive SPPT to breast milk sample B obtained after the mother had ingested peanuts, egg or cow's milk. IgE in the patient's serum recognized a group of proteins of approximately 14 and 30 kDa in the collected breast milk samples after the ingestion of peanuts, egg white or cow's milk, and all were inhibited with their respective allergens.

None of the infants had allergic symptoms after breastfeeding once their mothers started a specific allergen-free diet. Respect the other 42 children had consulted: 14 for erythema, 20 for vomiting and 18 for discomfort after breastfeeding. Only five

Table 1 *- Demographic and allergological data of the babies and their breastfeeding mothers.*

					SPPT breast milk mean diameter (mm)				
			Allergic History		A samples Collected 24 h after	B samples Collected (1-8 h) after ingesting			
case	**Age months**	**sex**	**baby**	**mother**	**a free diet of cow's milk, egg, peanut and hake**	**cow's milk**	**egg**	**peanut**	**hake**
1	10	F	AD	AD	0	NA	0	4.5	NA
2	14	F	AD wheezing	AD asthma	0	0	0	3	NA
3	3.5	F	AD	Asthma	0	0	3.2	0	0
4	8	M	AD	AD Asthma	0	0	2.5	0	0
5	15	F	AD wheezing	AD	0	NA	3.5	2.5	0

F (female), M (male), AD (atopic dermatitis). Results of SPPT babies with different breast milk A and B samples (mm average diameter); NA (not applicable, because of patient had a diagnosis of allergy to that food or patient is tolerating it at that time)

Table 2 *- Results of skin prick tests, total and specific IgE; tolerance status (yes, no, or not introduced) to cow's milk, white egg, hake and peanuts in each case.*

Case	Total IgE Ku/L	Cow's milk			White egg			Hake			Peanut		
		prick	**IgE**	**Tol**	**prick**	**IgE**	**Tol**	**prick**	**IgE**	**Tol**	**prick**	**IgE**	**Tol**
1	72	4.5	4.6	no	4	1.1	yes	0	0.0	NI	8	7.3	no
2	147	3	3.44	yes	3	4.35	NI	4	0.95	yes	6	31.9	no
3	9.22	0	0.00	yes	9	5.43	no	0	0.0	NI	0	0.0	NI
4	5.03	0	0.01	yes	4.5	0.1	no	0	0.0	NI	0	0.0	NI
5	92	13	25	no	6	12.9	NI	3	0.02	yes	6.5	0.36	no

of these patients had positive skin prick test, all to egg, but their symptoms had disappeared when we studied them. At this time their mothers were eating eggs without problems for their children.

Discussion

We studied infants who developed immediate allergic symptoms to human milk. We demonstrated that they had specific IgE to cow's milk, egg or peanut, none of which had been introduced into their diet. All infants had atopic dermatitis, which is a risk factor for food allergy, and two of them suffered also from asthma. Sensitization in the studied infants could have occurred in utero or through breast milk, inhalation, contamination of hands or household objects. Lactation has been suggested as being responsible for early sensitization. About 50% of women excreted dietary antigens in breast milk, concentrations ranging 0.1 to more than 1000 ng/ml (20). Characteristics of lactating women such as atopy have not accounted for the variable secretion (16,17,21). Ovalbumin has been detected in 59% to 74% (24, 25), bovine B-lactoglobulin in 53% to 63% (3,16,17) and peanut proteins in about 48% (9) of lactating women. Also ovomucoid (10,15), alpha-S1-casein (18), gliadin (19), and other food allergens have been detected in human milk under physiological conditions. Nevertheless, breast milk rarely triggers allergic symptoms and the role of food allergens in breast milk in food allergy in infants is not clear.

Two of our cases, 1 and 5, developed allergic symptoms after the first bottle of a cow's milk formula. Chandra et al. (1) found an incidence of cow's milk allergy of 0.5% (9/1749) in the first year of life with a frequency of exclusive breastfeeding of 52% at 3 months of age. Food allergens, other than cow's milk, could be responsible for allergic reactions in breastfed infants (15,21). Negative SPPT to breast milk samples obtained after a 24 h period of a cow's milk, egg or peanut-free diet and positive with samples collected after the ingestion of any of these foods and the results of immunoblotting experiments confirmed in each case the presence of hidden food allergens in the breast milk, justifying the appearance of symptoms when the babies were breastfeeding. Sera of the cases 1, 2 and 5, recognized in breast milk samples B (collected after mother ingestion of peanuts) 14 and 30 kDa allergens, this recognition was inhibited by previous incubation of sera with peanut. This/these 14 kDa allergen/s could correspond to Ara h 2, Ara h 5, Ara h 6, Ara h 7 or Ara h 10; and detected allergens of 30 kDa to an Ara h 3 fragment. Both major peanut allergens Ara h 1 and Ara h 2 have been previously identified in human milk by Vadas et al. (9) and Bernard et al. (22) who detected peanut allergens (Ara h 6) in human milk as early as 10 min after peanut ingestion, with peak values observed within the first hour after ingestion.

Sera of cases 3, 4 and 5 recognized, in breast milk samples B collected after egg ingestion, proteins around 14 KDa, possibly and between 20-30 kDa possibly Gal d 4 (lysozyme) and Gal d 1 (ovomucoid). Hirose et al. (23) detected ovomucoid in 12 out 37 (32%) human breast milk samples. Case 5 recognized cow's milk allergens between 14 to 30 kDa which could correspond to the Bos d 4, Bos d 5 and Bos d 8 (α-lactalbumin, β-lactoglobulin and caseins of cow's milk).

Böttcher et al. (26) and Järvinen et al. (27) observed an increased incidence of allergic disease in intentionally breastfed children. There is a prevailing opinion that breastfeeding decreases the allergy risk, and the mothers of high-risk infants might be more inclined to breastfeed than those of low-risk infants. Hong et al. (28) evaluated the effect of breastfeeding and gene-breastfeeding interactions in food sensitization in a birth cohort of 970 children, and observed that breastfeeding was associated with an increased risk of food sensitization; however, this effect was dependent on functional genetic variants in the IL-12 receptor b1, Toll-like receptor 9, and thymic stromal lymphopoietin genes. Liu et al. (29) evaluated a Boston birth cohort (n = 5,649) identifying a risk of sensitization for an IL4 gene polymorphism and 3 other genes.

Food allergens detected in breast milk could promote tolerance (30). Symptoms in our patients, during / after breastfeeding, disappeared when a cow's milk, egg or peanut maternal free diet was started. However, Case 1 had hen's white egg and Case 2 cow's milk, egg and fish IgE and both of them showed tolerance to maternal or direct ingestion of this potential allergens. Du Toit et al. (31) demonstrated a protective effect of peanut consumption during lactation by the proportion of UK Jewish mothers not consuming peanuts during breastfeeding, compared with Israeli Jewish mothers who ate peanuts, considering the ten times higher prevalence of peanut allergy in this population living in the UK. IgA in human milk might modulate mucosal immune processes and factors that promote gut maturation, such as intestinal microbiota, which could reduce allergy risk (32). Several breast milk peptides were found to lower regulate neonatal immune activity, suggesting they might promote neonatal immune competence. Järvinen et al. studied the role of maternal elimination diets and human milk IgA in the development of cow's milk allergy in the infants. They concluded that maternal CM avoidance was associated with lower levels of mucosal-specific IgA levels and the development of CMA in infants (33).

A consensus states that pregnant and breastfeeding women in general should not follow food allergen free diets (34,35). However, in case of allergic symptoms in breastfed babies, an allergic study should be performed to assessed food allergy.

Conclusion

Food allergens detected in breast milk could promote tolerance. A maternal free diet should be recommended only if food allergy is confirmed in breastfed babies.

Conflict of interests and funding

Authors declare that does not exist economic or other types of conflicts of interests and that the study did not receive funding

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Prevalence and associated factors for asthma in Brazilian and Japanese schoolchildren living in the city of São Paulo, Brazil

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Summary
Objectives. *Ethnic background interferes on the prevalence of asthma among schoolchildren (4 to 9 years old, SC) born and living in São Paulo, Brazil.* ***Methods.*** *International Study of Asthma and Allergy in Childhood (ISAAC)'s written standard and complementary questionnaires were applied to SC (similar socioeconomic status) living in the city of São Paulo: no-Japanese Brazilian (NJB, N = 306) and Japanese Brazilian (third generation, born in Brazil, from Japanese families with no miscegenation, JB, N = 258).* ***Results.*** *The prevalence of current asthma was significantly higher among NJB in comparison to JB (22.2% vs 14.7%, respectively). To have rhinitis and to exercise less than once/week were risk factors for both groups of children.* ***Conclusion.*** *Although both groups were apparently exposed to the same environment, other cultural differences do not allow us to conclude about the ethnic component having greater influence than the environment in the development of asthma in these individuals.*

Introduction

Despite overall improvements in health, there is renewed concern that racial and ethnic disparities in health persist and in some cases may have expanded. Ethnic health disparities are inherently linked to immigration because ethnic identities are traced to the country of origin of an immigrant or their ancestors. A body of international literature suggests that there is an increased prevalence of atopy and asthma in immigrants following migration (1-16).
Migration studies examining children of the same ethnic background living in different environments for part or all of their lives may help to identify relevant factors to the development of diseases and may explain some of the observed geographic variations in prevalence.
Leung et al. evaluated the prevalence of asthma and allergic diseases among Asian immigrants (Chinese) in Australia compared to non-Asian Australians and Asians born in Australia (1,2). They observed an increased prevalence of asthma among immigrants depending on time of immigration to Australia, regardless of age when immigrated, gender and atopic status, suggesting that the environment plays an important role in the pathogenesis of asthma and allergy (1,2). Similar results were observed by Wang et al.

in studying the prevalence of asthma among Canadian-born Chinese teenagers, young Chinese immigrants to Canada and young Chinese living in China, using data from the International Study of Asthma and Allergies in Childhood (ISAAC) phase 3. The prevalence of asthma among Chinese teenagers was lower than that of immigrants or those born in Canada, strengthening the influence of environmental factors on the prevalence of asthma (3). Other studies evaluated populations immigrated to Sweden (4), Italy (5-10), Israel (11), and United States of America (12-16).

In the last century, many people immigrated to Brazil, including the Japanese and nowadays they constitute the largest colony of Japanese individuals outside of Japan. The prevalence of current asthma in Brazil is 24.2% for children aged 6-7 years (17,18) while in Japan, it is 18.2% (18). This difference in prevalence opens the opportunity to evaluate the influence of environmental factors on ethnic factors, in relation to asthma.

Brazilian children of Japanese ancestry (i.e. no mixed marriages and whose grandparents were born in Japan), despite sharing genetic polymorphisms with those Japanese children, were born and live in a different environment: Brazil.

Migrating populations with no miscegenation provide an opportunity to observe changes in disease with changes in environment, just as genetically different groups living in the same region allow study of the effects of genetic diversity.

The aim of this study was to examine prevalence and risk factors for asthma manifestation among Brazilian children of Japanese ancestry and Brazilian children of non-Japanese ancestry period.

Materials and Methods

Children - children (aged 4 to 9) from three private schools - located in the southern part of the city of São Paulo, Brazil, and intended primarily for Japanese descendants (Japanese Brazilian, JB) - were invited to participate in this study. All schools were informed and acreddited by the Japanese Consulate in São Paulo. After an initial agreement by telephone, an interview was scheduled with the school's principal. Detailed explanation of the purpose of the study and the steps necessary to complete it correctly were provided (i.e. response to ISAAC written questionnaires (WQ) and informed consent signed by the parents or guardians). According to their origin children were divided into two ethnic groups, those born of marriages between Japanese only (third generation, born in Brazil, from Japanese families with no miscegenation; JB group) and those of non-Japanese Brazilian group (NJB). All students were from same socioeconomic level.

Sample size was calculated considering a α error of 5%, power of the test equal to 80% and 10% of difference in the prevalence of asthma (groups JB and NJB). So, the sample was estimated in 256 students in each group.

Questionnaires - ISAAC, standard (prevalence, phase 1, WQ) and complementary (risk factors - phase 2; CQ) written questionnaires, translated and validated for Brazilian culture (19,20) were applied according to the ISAAC protocol and answered by parents. The CQ was applied in order to evaluate the association between possible risk factors and asthma development and was answered by the parents at classroom.

The answers to the questions were transcribed to a database used by the ISAAC (Epi-Info 6.0), with double entry.

Statistical analysis - results were presented as percentage of affirmative responses among those applying. The comparative analysis between the two ethnic groups (JB and NJB) was performed by Chi-square test.

An affirmative answer to the question about wheeze in the last year and wheeze severe enough to limit speech in the last year defined SC with current asthma, and severe asthma respectively (18).

The groups of children with asthma symptoms and without asthma symptoms, in each ethinicity, were compared with respect to exposure to several factors identified by the CQ, and risk factors were identified by logistic regression. All variables from CQ were included in the univariate analysis and those with a $p < 0.20$ were included in the multivariate analysis and complemented with Forward stepwise regression. Variables with significant value were identified ($p < 0.05$).

Study was approved by the Ethics Committee of Federal University of São Paulo - Hospital São Paulo and all parents signed an informed consent.

Results

Table 1 shows the prevalence of asthma and related symptoms among NJB and JB children according to their progeny. The prevalence rates were in overal higher among NJB children. The prevalence of current asthma (22.2% *vs* 14.7%) and of wheezing with exercise (5.6% *vs* 1.9%) was significantly higher among NJB.

Table 2 shows the prevalence of asthmatic children exposed to some factors, during the first year of life and nowadays, and the comparison of the two progenies. NJB children were significantly more like to: be born by cesarean section, have dog and birds in the house nowadays, have dog and birds in the house during the first year of life, doing exercise less than once a week, eating fish once a week, have fruits and crude vegetables twice a week. The JB were significantly exposed to: being breastfeed equal or more than 6 months, attending day care/nursery, having father with rhinitis, sharing bedroom nowadays, and taking soft drinks twice a week (**table 2**).

All these factors were submitted to a multivariate analysis and to logistic regression (**tables 3**). To have rhinitis and to do exercise less than once a week remained as independent risk factors for current asthma among JB and NJB. To be a boy and to have shared the bedroom in the first year of life increased the risk for asthma manifestation only among JBs (**table 3**). To have eczema and have lived in an urban area during the first year of life was significantly associated with asthma only in NJB children.

***Table 1** - Prevalence of asthma and related symptoms among schoolchildren living in São Paulo, according to their progeny: Japanese Brazillian (JB) or non-Japanese Brazilian (NJB).*

Question	JB N = 258 (%)	NJB N = 306 (%)	OR (95% CI)
Wheezy ever	121 (46.9)	150 (49.0)	0.96 (0.80-1.14)
Wheezy last 12 months	38 (14.7)	68 (22.2)[1]	0.66 (0.42-0.95)[1]
More than 4 attacks last 12 months	6 (2.3)	5 (1.6)	1.42 (0.44-4.61)
Sleep disturbance last 12 months	22 (8.5)	41 (13.4)	0.64 (0.39-1.04)
Speech problem last 12 months	1 (0.4)	1 (0.3)	1.29 (0.81-2.05)
Asthma ever	20 (7.8)	21 (6.9)	1.22 (0.68-2.20)
Wheeze with exercise last 12m	5 (1.9)	17 (5.6)*	0.35 (0.13-0.93)[1]
Cough at night last 12 months	90 (34.9)	128 (41.8)	0.83 (0.67-1.03)

Chi-square/Fisher - [1]$p < 0.05$

Finally, owning a dog and live in an urban area was identified as protective factors for NJB (**table 3**).

Discussion

This study was performed to examine the prevalence of asthma and related symptoms, as well as to identify risk factors for asthma manifestation among Brazilian children of Japanese ancestry (JB) and Brazilian children of non-Japanese ancestry (NJB).

We observed lower prevalence of current asthma and related symptoms among JB born in Brazil, from non miscigenated marriages, in comparison to NJB students. However, our rates were close to those previously observed in Japan and obtained as part of the ISAAC phase III (18). Similar tendency was observed by other authors evaluating different immigrant populations (1,2,14,15,21). Indeed there are studies showing that the prevalence of asthma is lower among individuals who were not born in the country where the study was carried out. However, there are also evidences that the prevalence among immigrants, tends to match to that of the local population when enough time elapses (22).

So, regarding our findings, some questions remain with no answer: Would be the sample of JB schoolchildren evaluated by the ISAAC phase III in Japan representative of the country? Would be the ancestors of Japanese born in Brazil from the same locality of those Japanese who were evaluated in Japan? The lowest rate of interracial marriages would guarantee lower mixing of the JB population?

In fact, interactions among genetic, environmental and social factors seem to be crucial in determining the prevalence of asthma and asthma-related symptoms. Predictive factors for asthma vary among racial/ethnic groups (1-4,12). Identifying race/ethnicity-specific modifiable environmental and host-related factors can be important in developing targeted interventions to reduce the health and economic impact of asthma.

Given that the mixture of environment and genetic background may vary across racial/ethnic populations, in many instances it may be difficult to identify the causal genetic effect separately from the environmental one.

As already pointed out, migrating populations with stable genetics provide an opportunity to observe changes in disease due to changes in environment. The environment has unequivocally undergone changes over the past decades and it has been shown as an important risk factor associated mainly with westernized lifestyle (urbanization, lack of exercise, dietary patterns, air pollution, and indoor pollution by passive smoking and aeroallergens, improved hygiene and health care etc. (23,24).

To be born and to live in the same locality give us the wrong idea that our children, JB and NJB, would be exposed to the same environmental factors. However, we must take into account the importance of cultural factors that may significantly influence lifestyle. This fact becomes clear when evaluating the differences on exposures observed between JB and NJB. Significant differences ocurred with respect to: type of delivery, duration of exclusive breastfeeding, type of feeding, having pets personal and family history of allergic diseases. We believed that some of these differences could be explained mainly by the maintenance of very ingrained habits in Japanese culture.

However, after logistic regression we observed some of our findings were unexpected. First of all we found that it remained as independent risk factors for current asthma, among JB and NJB, to have rhinitis and lack of exercise (less than once a week). Recent meta-analysis evaluated the prevalence and interrelationships between asthma, allergic rhinitis and eczema in children using data obtained from ISAAC questionnaires. The analisys has shown that the prevalence of children with a co-occurrence of asthma, eczema and allergic rhinitis was low, but significantly higher than could be expected by chance (25).

Table 2 *- Factors associated to asthma manifestation identified by univariate analysis among children aged from 4 to 9 years according their progeny - comparisons between Japanese Brazilian (JB) and No-Japanese Brazilian (NJB).*

Associated factors	**NJB**			**JB**		
	N total	**N +ve**	**%**	**N total**	**N+ve**	**%**
Birth weight < 2500g	302	33	10.9	258	38	14.7
Cesarean section	290	247	*85.2*[1]	246	170	69.0
Be twin	298	8	2.7	254	4	1.6
Breast feeding	304	282	92.8	258	244	94.6
Breastfeeding ≥ 6 months	282	167	59.2	246	188	*76.4*[1]
Breastfeeding ≥ 4 months	282	98	34.8	246	116	*47.2*[1]
To have older brothers	304	157	51.6	258	145	56.2
To have younger brothers	303	87	28.7	254	99	39.0
Day care / nursery	303	53	17.5	254	88	*34.6*[1]
Day care / nursery ≤ 1st year	53	31	58.5	88	63	71.6
Kind garden	284	284	100	241	240	99.6
Kind garden ≤ 1st year	283	14	4.9	250	21	8.4
Mother with asthma	306	15	4.9	258	5	1.9
Mother with rhinitis	306	110	35.9	258	88	34.1
Mother with eczema	306	19	6.2	258	25	9.7
Father with asthma	306	26	8.5	258	11	4.3
Father with rhinitis	306	84	27.5	258	78	*30.2*[1]
Father with eczema	306	13	4.3	258	19	7.4
Share bedroom today	293	163	55.6	252	176	*69.8*[1]
Share bedroom 1st year	244	139	57.0	231	147	63.6
Dog in the home today	306	96	*31.4*[1]	258	44	17.1
Cat in the home today	306	16	5.2	258	8	3.1
Birds in home today	306	53	*17.3*[1]	258	20	7.8
Dog in home 1st year	306	43	*14.1*[1]	258	18	7.0
Cat in home 1st year	306	9	2.9	258	3	1.2
Birds in home 1st year	306	26	*8.5*[1]	258	1	0.4
Smoking mother	294	31	10.5	253	17	67.2
Smoking mother 1st year	253	24	9.5	236	17	64.6
Smoking during pregnancy	253	21	8.3	224	10	4.5
Smoking in the house	303	41	13.5	256	32	12.5
Damp in home today	302	36	11.9	257	43	16.7
Damp in home 1st year	302	35	11.6	235	29	12.3
Mold today	300	27	9.0	257	22	8.6
Mold 1st year	259	25	9.7	239	17	7.1
Rural neighborhood today	278	57	20.5	248	41	16.5
Rural neighborhood 1st yr	236	46	19.5	232	58	25.0
Exercise less than once / week	298	241	*80.9*[1]	256	185	72.3
Eat meat twice a week	300	88	29.3	253	67	26.5
Eat fish once / week	286	258	*90.2*[1]	254	178	70.0
Fruits twice / week	294	103	*35.0*[1]	257	66	25.7
Crude vegetables twice / week	294	182	*61.9*[1]	254	89	35.0
Soft drink twice / week	299	195	65.2	255	208	*81.6*[1]

[1]Chi-square - Values in italic bold were $p < 0.05$; +ve = positive

Table 3 *- Factors associated with symptoms of asthma among children aged from 4 to 9 years, according to their progeny: Japanese Brazilian (JB) or No-Japanese Brazilian (NJB) identified by Logistic regression.*

Associated factors	**JB**	**NJB**
	OR (95% CI)	**OR (95% CI)**
Have cat nowadays	5.60 (0.89-35.33)	-
Urban neighborhood 1st year	-	5.32 (1.51-18.78)[1]
Have rhinitis	3.71 (1.69-8.14)[1]	2.85 (1.53-5.32)[1]
Male gender	2.96 (1.34-6.57)[1]	-
Have eczema	-	2.39 (1.06-5.40)[1]
Father with asthma	-	2.39 (0.95-6.06)
Exercise less than once / week	2.36 (1.04-5.34)[1]	2.01(1.00-4.03)[1]
Share bedroom 1st year	2.20 (1.00-4.87)[1]	-
Have dog nowadays	-	0.47 (0.23-0.93)[1]
Urban neighborhood nowadays	-	0.16 (0.05-0.49)[1]
Father with eczema	0.16 (0.02-1.32)	-

[1]$p < 0.05$; - = not included in the analysis

There is no doubt that a sthma and rhinitis should be viewed as a single disease, considering the high frequency of association between them. The presence of allergic rhinitis was significantly associated with current asthma in both groups. This fact, amply reported by other authors (26,27) had been previously documented by our group in schoolchildren assessed by the ISAAC phase III when we observe that to have active rhinitis increased significantly the risk of active asthma, and severe asthma in those schoolchildren (28).

We found that JB and NJB children who exercised less than once a week had a risk 2.36 and 2.01 times higher, respectively, to manifest asthma. In this regard, several studies suggest that nutrients (e.g. omega-3 fatty acids, vitamin D) and consumption of fruits and vegetables protect against asthma, while obesity and lack of exercise could have the opposite effect (29-31). The links that exist between asthma and obesity suggest that obesity probably leads to asthma in many cases and could be in part responsible for the "asthma epidemic". Moreover, there are two other very important factors - diet and exercise - which can favour both asthma and obesity in parallel. There is a growing body of literature that implicates specifically decreased physical activity, as a contributor to the increase in asthma prevalence and severity. Although the prevalence of asthma and related symptoms in our study has been lower among the JB, there were no differences regarding severity which remained intense in both groups (32,33).

To live in an urban area in the first year of life was significantly associated with asthma in NJB children. Intriguingly, among JB this effect was not observed, while the exposure to urban environment - nowadays - indeed protected NJB from asthma. Asthma prevalence and morbidity use to be greater in urban areas. Despite the number of studies looking for information about the relationship between early life exposures and asthma in this "high-risk environment" this association remain not completely clear and the doubts persist.

The urban environment has a number of features that could have adverse effects on children's respiratory health, especially during the first few years of life when the lung and immune system are rapidly developing (34).

However, there are limitations in ecological studies due to the difficulty in accurately estimating specific exposure - individual or combined - to infer a cause-effect (35-37). So the specific factors or combinations of factors that lead to asthma and/or protect against asthma when analising the indoor and outdoor environment, remains not completely elucidated.

It is quite possible that the differences between the indoor environment of JB and NJB can explain our observations (i.e. no association between urban neighborhood over the 1st year and asthma manifestation, among JB). In other words, perhaps differences in customs and habits - between JB and NJB - account for differences in indoor environment and consequently in exposures.

On the other hand although, there is evidence suggesting that exacerbations of asthma may be triggered by different air pollutants, the association between air pollution and increased prevalence of asthma is still controversial (38). Maybe this association would be clearer during the first year of life for the NJB children and not detectable nowadays. We found that to have eczema

increased in almost 2.4 fold the risk of asthma among NJB children. Intriguingly, we did not observe this association among JB. Whether eczema is a true risk factor for asthma has been debated, and the relationship between the different allergy-related disorders is unclear. There are evidences from cross-sectional and also from large prospective studies, indicating a strong association between eczema and asthma (28) period.

However, it is noteworthy that in addition to the early manifestation of eczema, it is commonly believed that the severity of eczema, male sex, early wheezing, heredity and allergic sensitisation are possible risk factors for the development of childhood asthma (39).

To be a boy and to have shared the bedroom in the first year of life increased the risk for asthma manifestation only among JBs. We can not explain why the classical association "male gender and increased risk for asthma" did not occur among NJB. Gender seems to be an important determinant for asthma and allergies and its impact varies considerably from childhood into adolescence and adulthood. In childhood, boys are consistently found to be at increased risk of asthma, which has been explained by differential growth of lung/airway size, and immunological differences (40,41).

The hygiene hypothesis suggests a protective effect of large families, with many children living in the same environment and often sharing the same bed. This kind of environmente favours viral infecctions and may increase the exposure to endotoxins, and these factors may protect the child from sensitization to aeroallergens, asthma and rhinitis.

A number of epidemiological studies, using different measures of crowding such as total number of residents in the home, number of siblings, number of persons sharing the bed, room occupancy, and population density, have reported an association between crowding and respiratory diseases (42).

There are studies demonstrating an inverse relationship between the number of people in the bedroom and the frequency of asthma (protection) (42) and others an association between sharing a bedroom during the first year of life and asthma (risk) (43).

Unlike one of the proposals of the hygiene hypothesis, we found that sharing a bedroom during the first year of life was a risk factor, not a protective factor for current asthma among JB.

In this study, the association between exposures to pets at home was different depending on ethnicity. Among JB, to have been exposed to a cat or a dog during the first year of life or nowadays did not exert any significant effect on asthma manifestation. On the other hand to be exposed nowadays to dogs protects NJB from the disease. The role of pet exposure, mainly cat and dog, inside the house as either a risk or a protection factor for childhood asthma manifestation is still controversial. Exposure to cats and dogs at home was evaluated in children aged 6-7 years and adolescents (13-14 years) participating in the ISAAC phase 3. Early-life exposure to cats was identified as a risk factor for symptoms of asthma, rhinoconjunctivitis, and eczema in 6-7-year-old children, especially in less-affluent countries. Current exposure to cats and dogs combined, and only to dogs, is a risk factor for symptom reporting by 13-14-year-old adolescents worldwide (44) period.

There are lots of evidence about the strong genetic component on allergic diseases and asthma manifestation. In this respect, maternal and paternal history of asthma and allergic diseases has been consistently implicated on a higher risk of childhood asthma, on the great majority of studies. Maternal history of asthma seems to has greater impact on the subsequent development of asthma in children, than paternal history of asthma.

Surprisingly, we did not observe a significant relationship between family history of allergic diseases and asthma manifestation neither among JB nor NJB. We can not explain this finding. Even knowing that this type of study could have led to memory bias, we didn't believe that these items could explain the "absence of the role of maternal and paternal history on the risk of asthma development".

In conclusion, we observed lower prevalence of current asthma and related symptoms among JB born in Brazil, from non miscigenated marriages, in comparison to NJB students.

Different factors were implicated on the risk of asthma depending on ethnicity/race, except "to have rhinitis" and "lack of exercise" that were independent risk factors for current asthma among JB and NJB. "Owning a dog" and "living in an urban area" were the only protective factors identified just among NJB children.

It is known that genetically similar populations exposed to different environmental conditions display different temporal trends in the prevalence of allergic symptoms. However, because of the interaction and of the multiple causal pathways between the factors studied, the exact contribution or the exact influence that each one it would have exerted on differences and similarities observed - according to race/etinicity - is very difficult to establish and to validate.

Further studies in Brazil and Japan, including objective measures such as allergen skin prick test, bronchial hyperresponsiveness and environmental measurements (e.g., endotoxins and diesel exhaust particles) are necessary to identify the risk factors or protective factors associated with asthma.

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Patients with breakthrough reactions to iodinated contrast media have low incidence of positive skin tests

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Key words
skin tests; hypersensitivity; iodinated contrast media; breakthrough reactions; intradermal tests

List of abbreviations used:
ICM iodinated low-osmolality contrast media; ST skin test; SPT skin prick tests; IDT intradermal tests; PT patch tests; IR immediate reaction; NIR non-immediate reaction.

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Summary
Background. *The term "breakthrough reactions" designates repeated hypersensitivity reactions to iodinated contrast media (ICM) despite premedication with glucocorticoids and antihistamines. We aimed to retrospectively evaluate the rate of positive skin test (STs) in our cohort of patients with previous breakthrough reactions to different ICMs.* ***Methods.*** *A series of 35 patients, who experienced at least one breakthrough reaction to ICM and who underwent STs within 6 months from the reaction were studied, and results were compared to a control group of patients with a first hypersensitivity reaction occurred without premedication. Skin prick tests (SPT), intradermal tests (IDT) and patch tests (PT) at different dilutions, with a set of three to four ICM were performed.* ***Results.*** *Of the 35 patients with prior breakthrough reactions, 57% had an immediate reaction (IR) and 43% had a non-immediate reaction (NIR). Patients who experienced the first hypersensitivity IR or NIR, later had one or more breakthrough IR or NIR, respectively. Overall, 29% (10/35) of patients with prior breakthrough reactions resulted positive to STs compared to 57% (16/28) of the control group ($p < 0.05$). No significant difference in allergy history, age, sex, other clinical / demographic features nor chronic use of ACE-inhibitor, beta-blockers or NSAIDs was observed.* ***Conclusions.*** *This preliminary finding suggests that patients with prior breakthrough reactions have significantly lower immunologically proven ICM reactions (positive STs) if compared to non-breakthrough patients. According to that, a considerable number of breakthrough reactions seems to be non-allergic hypersensitivity reactions or reactions which could be mostly prevented by a proper, well-timed skin testing. Larger prospective studies are needed to confirm these results, with a more careful analysis of patients' risk factors, a laboratory assessment that includes an in vitro allergy diagnostics, and hopefully a drug provocation test for selected cases.*

Introduction

The term "breakthrough reactions" refers to repeated hypersensitivity reactions to iodinated contrast media (ICM), nowadays non-ionic, low-osmolality contrast media, despite premedication with glucocorticoids and antihistamines (1-4). A large amount of literature has been formerly written on this topic, when immunological mechanisms beyond hypersensitivity reactions were quite neglected (1-6). In fact, breakthrough reactions were often described without distinguishing if occurring after injection of the same rather than a different ICM responsible of the prior reaction (1,3,4). From a clinical perspective, patients with breakthrough hypersensitivity reactions to ICM are often patients who undergo and require many contrast-enhanced examinations, such as patients with oncologic or cardiovascular diseases. Thus, the

selection of a safe alternative compound is fundamental and starts from the demonstration of the patient's sensitization to one or more ICM assessed by skin tests (STs) (7-10).

The reliability of STs in diagnosis of ICM allergy has already been assessed in patients with prior hypersensitivity reaction to ICM (10-14). In particular, ENDA conducted a prospective multicenter study which demonstrated that a diagnosis could be reached in up to 50% of patients with prior hypersensitivity reactions to ICM if tested by STs (namely skin prick tests, intradermal tests and patch tests) between 2 and 6 months after the reaction (10).

Since true sensitivity of STs in patients with prior breakthrough reactions are still unknown, we retrospectively analyzed the rate of positive STs performed within 6 months after (the last) breakthrough reaction in these patients and compared this data to a control group that experienced a hypersensitivity reaction to ICM without premedication.

Material and methods

Patients. Data of patients who had one or more hypersensitivity reactions to ICM despite pharmacological premedication in our Radiology Department between December 2006 and December 2014 were collected. Patient demographics; risk factors; ICM culprits; signs, symptoms, severity and timing of each index and breakthrough reaction were reported, as suggested by ENDA questionnaire for drug hypersensitivity (15). We also included patients who experienced breakthrough reactions in our Radiology Department, but who experienced the first hypersensitivity reaction to ICM (the one occurred without premedication, also called *index* reaction) elsewhere, only if the ICM of the index reaction was known. A total of 35 patients was collected.

We compared STs results with a control group of patients who had a hypersensitivity reaction to ICM without pharmacological premedication in our Radiology Department between January and December 2014, which were tested with STs at the same conditions of the breakthrough patients' group. Data of 28 patients were collected.

Written informed consent was obtained for the procedure. No ethical committee approval was requested for this observational analysis, since all tests are already accepted as routine tools and were performed for diagnostic purposes.

Skin tests and contrast media. We included only those patients who were tested by skin prick test (SPT) and intradermal test (IDT) with a set of three ICM (iomeprol, iopromide, iodixanol), between 2 and 6 months from the breakthrough reaction (or the last breakthrough reaction if more than one) (10,13). Patch test (PT) were performed only in patients with non-immediate reactions (10). Given the retrospective nature of the study and its purpose, patients with STs performed after *index* reaction and before breakthrough reaction in study cohort were not included. Similarly, STs were performed between 2 and 6 months from the hypersensitivity reaction in the control group. Iomeprol (Iomeron 350 mg/mL), iopromide (Ultravist 370 mg/mL) and iodixanol (Visipaque 320 mg/mL) were chosen for STs, being the only ones used in our center for radiological examinations in the last 8 years (CT-scan, conventional angiography, cholangiography and urography) (11). Hence, if a patient experienced a hypersensitivity reaction to ICM in our Radiology Department in this time interval, the culprit should be searched among one of this three. For those patients who experienced the *index* reaction elsewhere, we added the culprit compound, if different from these three, to the aforementioned panel used for STs.

SPT were performed with undiluted commercially available ICM solution, IDT was administered at gradually increasing concentrations of 1:100, 1:10 and then 1:1 dilutions, whereas PTs were performed with undiluted ICM and all the results were interpreted according to the International Guidelines and the ENDA study protocol (10,13). SPT and IDT were evaluated after 20 minutes (immediate reading), while PT and IDT were evaluated after 48, 72 and 96h (delayed reading).

Reaction time and severity. As previously described by our group (11), Hypersensitivity reaction were divided according to the time between ICM injection and reaction onset. Immediate reactions (IRs) were defined as those developing within one hour after ICM injection, whereas non-immediate (or delayed-type) reactions (NIRs) as those developing from one hour to one week after contrast media administration (9). Immediate reactions were assessed according to the Ring and Messmer classification from grade 1 to 4 as follow: grade 1 for generalized cutaneous and/or mucocutaneous rash, skin eruption, urticarial, angioedema and pruritus; grade 2 for mild systemic reactions including skin manifestations, abdominal symptoms (nausea, cramping), respiratory symptoms (rhinorrhea, hoarseness, dyspnea), cardiovascular symptoms (tachycardia $\Delta > 20$ / min); grade 3 for life-threatening systemic reactions including abdominal symptoms (vomiting, diarrhea), respiratory symptoms (laryngeal edema, bronchospasm, cyanosis), cardiovascular symptoms (hypotension > 20 mmHg sys., arrhythmia, shock); and grade 4 for cardiac and/or respiratory arrest (16). Non-immediate reactions were defined as mild when no treatment was required, moderate when the patient responded quickly to an appropriate treatment (e.g. oral glucocorticoid), and severe when the reaction was life-threatening, required hospitalization or resulted in death (9).

Premedication regimen. All the patients were premedicated with the same regimen of corticosteroids and antihistamine before undergoing the radiological procedure, as already described in other study-cohort (11). Briefly, the premedication regimen used in our center is approved and adopted by the American

College of Radiology (8): Methylprednisolone (Medrol®) 32 mg by mouth 12 hours and 2 hours before ICM administration and Hydroxyzine Hydrochloride (Atarax®) - 25 mg by mouth 1 hour before ICM administration.

Statistical Analysis. Continuous variables are expressed as average (range minimum-maximum value), unless otherwise specified. Qualitative data were expressed in frequency and percent. Fisher's exact test and Student's T test were used for statistical comparison between groups. Differences with P-values below of 0.05 were considered statistically significant.

Results

Patients' features. We identified 58 patients with prior breakthrough hypersensitivity reactions, but 23 did not fulfilled the study criteria (STs were performed after 6 months form the least breakthrough reaction). A total of 38 hypersensitivity reactions to ICM despite pharmacological premedication occurred in 35 patients (mean age 58 years, range 26-78). The 57% (20/35) of patients had an *index* IR, whereas the 43% (15/35) of patients had an *index* NIR. All *index* IRs and NIRs were subsequently followed by one or more breakthrough IRs and NIRs respectively (**table 1**).

Sixteen patients (46%; 8 patients of the IR group and 8 of the NIR group) reported a history of previous hypersensitivity reactions to agents different from ICM. In particular, drug hypersensitivity was the majority of cases (75%), half of which were severe (50%). The 66% (13 patients with IR and 10 with NIR) had a positive history for oncologic diseases, most frequently lymphoma (35%). The 9% (3/35) of patients had chronic obstructive pulmonary disease, the 17% (6/35) had coronary artery disease and the 6% (2/35) had a systemic autoimmune disease.

Clinical features of the 28 patients of the control group, who experienced a hypersensitivity reaction without any premedication, were comparable with the patients of the breakthrough group regarding distribution of background characteristics including age, gender, history of allergic disease, comorbidities, NIRs / IRs distribution, and severity of hypersensitivity reactions (**table 1**).

ICM used and radiological examinations. Among patients cohort with prior breakthrough reactions, most of radiological examinations of hypersensitivity reaction occurred without pharmacological premedication, called *index* reaction, were CT scan (33/38); 2 were conventional angiography, 2 were cholangiography and 1 was urography. All the radiological examinations of breakthrough reaction of this group were CT scans. Similarly, all radiological examinations performed in the control group were CT-scans.

Among the cohort of patients with prior breakthrough reactions, the ICM of the *index* reaction was known in 18/35 patients (51%; **table 1** for details). Three out of 35 patients had more than one breakthrough reaction, for a total of 38 breakthrough reactions. In 34 out of 38 breakthrough reactions ICM culprit were known (89% of patients); while in the control group, ICM culprit was known in 100% of cases (**table 1** for details).

Hypersensitivity reactions' severity. Among patients of the breakthrough group, IRs were experienced in 20 and NIRs in 15. Of note, each index IR was followed by one or more IRs and each NIR was followed by one or more NIRs. Among *index* IRs, 45% were assessed as grade I, 20% as grade II, and 35% as grade III, whereas among *index* NIRs, 87% were graded as mild and 13% as moderate reactions. Among breakthrough IRs, 48% was grade I and 52% grade II and among breakthrough NIRs, only 73% were considered mild and 27% moderate reactions. In the control group, 53% of IRs were assessed as grade I, 29% as grade II and 18% as grade III. Almost three quarters of NIRs were considered mild, 27% moderate and none severe (**table 1**).

Patients with prior breakthrough reactions have low rate of positive ST. All patients tested presented a histamine wheal ≥ 3 mm. Overall, 29% (10/35) of patients with prior breakthrough reactions had positive STs to one or more ICM tested. Among these 10 patients, 6 had a prior IR and 4 had a prior NIR. Details are reported in **table 2** and **table 3**. In the control group, the rate of positive STs to one or more ICM tested was 57% (16/28). Among these 16 patients, 10 had a prior IR 6 had a prior NIR. The difference between breakthrough and control groups was statistically significant ($P < 0.05$). There was no difference in STs positive rate comparing each other IR subsets of breakthrough and control group and NIR subsets of breakthrough and control group ($p > 0.05$ in both comparisons).

In the cohort of patients with prior breakthrough reactions, none of the patients had positive SPT, whereas 9/10 patients had positive IDT (6 IR patients and 3 NIR patients) and one of the NIRs group had also positive PT only (**table 2** and **3** for details). The median time interval between the first reaction and skin testing was 5 months (range 2 - 6).

The culprit ICM of the breakthrough reaction (or the last breakthrough reaction if more than one) was known in 33 out of 35 patients, 19 with IRs and 14 with NIRs. The culprit ICM elicited a positive ST in 26% (5/19) of IR patients and in 21% (3/14) of NIR patients. As mentioned, all STs were performed within 6 months from the last breakthrough reaction. The culprit ICM of *index* reaction (occurred from 6 months to 8 years before STs) of the breakthrough cohort, was known in 51% (18/35) of patients, 10 with IRs and 8 with NIRs. Three of them tested positive to the implicated ICM, 20% (2/10) of IR patients and 13% (1/8) of NIRs. In 2 of the 3 patients of IR group who experienced two breakthrough reactions each, the ICM used of both breakthrough reactions were the same and in one the ICM was unknown (**table 2**).

In the control group, none of the patients had positive SPT or PT, whereas 16/16 patients had positive IDT (10 IR patients

Table 1 *- Clinical features of patients studied.*

	Breakthrough group	**Control group**
Number of Patients	35	28
Female, n (%)	27 (77%)	21 (75%)
Age, mean (range)	58 (26-78)	60 (28-74)
Immediate / Non-Immediate Reaction	20/15	17/11
Allergic history, n (%)	16 (46%)	14 (50%)
Other drug allergies	12	10
Common inhalants	5	4
Hymenoptera venom	1	0
Gadolinium	1	0
Comorbidities, n (%)		
Oncological disease	23 (66%)	17 (61%)
Chronic pulmonary disease	3 (9%)	2 (7%)
Coronary artery diseases	5 (17%)	2 (7%)
Autoimmune disease	2 (6%)	0
Chronic use of ACE-I or NSAIDs, n (%)	12 (34%)	10 (36%)
ACE inhibitor	3	2
NSAIDs	4	3
Beta blockers	5	5
ICM of index reaction, n (%)	35	28
Iopromide (non-ionic monomer)	5 (14%)	9 (32%)
Iomeprol (non-ionic monomer)	5 (14%)	11 (39%)
Iodixanol (non-ionic dimer)	4 (11%)	8 (29%)
Iopamidol (non-ionic monomer)	2 (6%)	0
Severity of index reaction, n (%)		
Grade I	9 (45% of IR)	9 (53% of IR)
Grade II	4 (20% of IR)	5 (29% of IR)
Grade III	7 (35% of IR)	3 (18% of IR)
Grade IV	-	-
Mild	13 (87%of NIR)	8 (73% of NIR)
Moderate	2 (13% of NIR)	3 (27% of NIR)
Severe	-	-
ICM of breakthrough reaction, n (%)	38	-
Iopromide (non-ionic monomer)	21 (60%)	-
Iomeprol (non-ionic monomer)	8 (23%)	-
Iodixanol (non-ionic dimer)	6 (17%)	-
Unknown	4 (11%)	
Severity of breakthrough reaction, n (%)		
Grade I	11 (48%)	-
Grade II	12 (52%)	-
Grade III	-	-
Grade IV	-	-
Mild	11 (73%)	-
Moderate	4 (27%)	-
Severe	-	-

Table 2 *- Skin testing for patient with IR to ICM.*

Pt.	Index reaction		Prior breakthrough reaction(s)		Last breakthrough reaction		Skin test results[1]
	Severity	ICM	Severity	ICM	Severity	ICM	
1	grade III	Unknown	-	-	grade II	Iopromide	-
2	grade III	Unknown	grade II	Unknown	grade I	Iomeprol	IDT 1:100 Iomeprol (I, 96) and 1:100 Iopromide (I, 96)
3	grade III	Unknown	-	-	grade II	Unknown	-
4	grade I	Unknown	-	-	grade II	Iopromide	-
5	grade I	Unknown	-	-	grade I	Iopromide	IDT 1:10 Iopromide (I) and 1:1 Iomeprol (I, 96)
6	grade III	Unknown			grade II	Iopromide	IDT 1:100 Iopromide (I)
7	grade III	Unknown	grade I	Iomeprol	grade I	Iomeprol	-
8	grade I	Unknown	-	-	grade I	Unknown	-
9	grade I	Unknown	-	-	grade I	Iopromide	-
10	grade I	Unknown	-	-	grade I	Iopromide	IDT 1:100 Iopromide (I)
11	grade I	Ioversol	-	-	grade I	Iomeprol	-
12	grade III	Iopromide	-	-	grade II	Iodixanol	IDT 1:10 Iopromide (I), 1:1 Iomeprol (I) and 1:1 Iodixanol (I)
13	grade II	Iodixanol	-	-	grade II	Iopromide	-
14	grade III	Iopamidol	-	-	grade II	Iopromide	-
15	grade II	Iomeprol	-	-	grade II	Iodixanol	IDT 1:100 Iomeprol (I) and 1:100 Iopromide (I)
16	grade I	Iomeprol	-	-	grade I	Iopromide	-
17	grade I	Iodixanol	grade I	Iopromide	grade I	Iopromide	-
18	grade II	Iopamidol	-	-	grade II	Iopromide	-
19	grade II	Iodixanol	-	-	grade II	Iodixanol	-
20	grade I	Iohexol	-	-	grade II	Iodixanol	-

[1]Skin tests included SPTs and IDTs: only positive results are reported. Only 3 patients had two consecutive breakthrough reaction.
Computed tomography (CT), iodinated contrast media (ICM), Immediate reaction (IR), Intradermal test (IDT), immediate reading (I), 48 hours reading (48), 72 hours reading (72), 96 hours reading (96).

and 6 NIR patients, **table 2-3** for details). The STs positive ICM matched the culprit ICM in 3 patients of the IR group and in 2 patients of the NIR group, respectively. The median time interval between the first reaction and skin testing was 4 months (range 2 - 6).

No difference was observed in clinical/demographic features or chronic ACE-inhibitor / beta-blockers / NSAIDs use between breakthrough and control groups.

In both groups the rate of chronic use of ACE-inhibitor and/or beta-blockers and/or NSAIDs was more than 30% (**table**

Table 3 *- Skin testing for patient with NIR to ICM.*

Pt.	Index reaction		Last breakthrough reaction		Skin test results[1]
	Severity	**ICM**	**Severity**	**ICM**	**-**
1	Mild	Unknown	Mild	Iomeprol	-
2	Mild	Unknown	Mild	Iomeprol	-
3	Moderate	Unknown	Moderate	Iopromide	IDT 1:10 Iomeprol (72) and 1:10 Iopromide (72)
4	Mild	Unknown	Mild	Iopromide	-
5	Mild	Unknown	Moderate	Iopromide	-
6	Mild	Unknown	Mild	Iodixanol	IDT 1:10 Iodixanol (96)
7	Mild	Iodixanol	Mild	Iopromide	IDT 1:100 Iopromide (I) and 1:1 Iodixanol (I)
8	Mild	Iomeprol	Moderate	Iopromide	-
9	Mild	Iopromide	Moderate	Iomeprol	-
10	Moderate	Iomeprol	Mild	Iopromide	-
11	Mild	Iopromide	Mild	Iopromide	-
12	Mild	Iopromide	Mild	Iopromide	-
13	Mild	Iomeprol	Mild	Iomeprol	-
14	Mild	Unknown	Mild	Unknown	PT iodixanol (48)
15	Mild	Iopromide	Mild	Iopromide	-

[1]Skin tests included SPTs, IDTs and PTs: only positive results are reported.
Computed tomography (CT), iodinated contrast media (ICM), Non-immediate reaction (NIR), Intradermal test (IDT), Patch test (PT), immediate reading (I), 48 hours reading (48), 72 hours reading (72), 96 hours reading (96).

Table 4 *- Clinical/demographic features and ACE-inhibitor / NSAIDs /Beta blockers chronic use in patients with positive ST's results.*

	Breakthrough group with STs +	**Control group with STs +**	**P**
Age	57	64	> .05
Female	(8/10) 80%	(12/16) 75%	> .05
Allergy history, n (%)	5/10 (50%)	9/16 (56%)	> .05
Other drug allergies	4	7	
Common inhalants	1	2	
Comorbidities, n (%)	8/10 (80%)	11/16 (69%)	> .05
Oncological disease, n (%)	6/10 (60%)	9/16 (56%)	
Chronic pulmonary disease, n (%)	1/10 (10%)	-	
Coronary artery diseases, n (%)	1/10 (10%)	2/16 (13%)	
Chronic use of ACE-I or NSAIDs, n (%)	4/10 (40%)	6/16 (38%)	> .05
ACE inhibitor	2	2	
NSAIDs	1	3	
Beta-blockers	1	1	

4). Among the patients who had prior breakthrough reactions with positive STs (10 patients), 40% chronically used medications potentially exacerbating a ICM hypersensitivity reaction (ACE-inhibitor 2 patients, NSAIDs 1 patient, beta blockers 1 patient). In the control group, 38% (n = 16) of patients with positive STs chronically used these medications (ACE-inhibitor 2 patients, NSAIDs 3 patients, beta blockers 1 patients). There was no statistical difference between the breakthrough and the control group of patients. Results are summarized in **table 4**.

Furthermore, there was any significant difference in allergy history, age, sex and other demographic features in STs-positive subsets of both groups (**table 4**).

Discussion

The problem of repeated hypersensitivity reactions to ICM despite premedications, formerly called breakthrough reactions, represent a major issue in clinical setting if a new contrast-enhanced radiological examination is required. From a clinical perspective, the diagnosis by STs of the ICM culprit (if not known) or other cross-reactive ICMs is the prerequisite for selection of an alternative compound and prevention of a possible new reaction (7-10). Overall, the use of STs has not yet been assessed in patients with prior breakthrough reactions (occurred despite premedication), whereas a growing body of literature reported the sensitivity of these testing around 50% for patients who experienced ICM hypersensitivity reaction (10-14).

In the present study we aimed to retrospectively evaluate the rate of positive skin test (STs) in 35 patients with previous ICM breakthrough reactions, and to compare this results to a control group of patients who experienced an ICM hypersensitivity reaction occurred without premedication.

We included only those patients in which STs were performed within 6 months from the last breakthrough reaction, in order to optimize the rate of positive testing. Interestingly, we found that the STs were positive in 29% (10/35) of patient's cohort with prior breakthrough reactions, equally distributed between IR group and NIR groups, versus 57% (16/28) of the non-breakthrough control group ($p < 0.05$, **table 2** and **3**). Of note, the STs rate of the control group was consistent to those already published (10).

Overall, less than one third of patients with prior breakthrough reactions has immunologically proven ICM reactions (with positive STs). A possible explanation to this unexpected result may likely have been the unintentional selection of the population studied. In fact, patients with repeated reactions despite premedication are usually patients who undergo to several contrast-enhanced radiological examination because of an oncologic or cardiovascular disease, unlike the ENDA patients' cohort. Nevertheless, no significant difference was found in epidemiological and clinical features between the breakthrough patients' cohort and control group. Similarly, the chronic use of ACE-inhibitor and/or beta-blockers and/or NSAIDs, which may potentially trigger or exacerbate an ICM reaction, was not increased in the breakthrough group (**table 4**).

However, a deeper reading of the results achieved, evidences that most of the so called "breakthrough reactors" with negative STs are likely patients with non-allergic breakthrough reactions. Overall, 29% of patients are positive at STs for one or more ICM, and only 26% of IR and 21% of NIR patients are ST positive for the breakthrough reaction's ICM. This suggest that the majority breakthrough reactions are probably non-immunologic reactions, due e.g. to direct histamine release by circulating basophils or even steroid-induced flushing. Our clinical experience supports this view, particularly for the breakthrough IRs. On the other hand, only 20% of IR and 13% of NIR patients are ST positive for the index reaction's ICM (from 6 months to 8 years before STs), paralleling the data already published by ENDA group for patients tested after 6 months from the hypersensitivity reaction, in which ST positive rate was around 18% for IR and 22% for NIR respectively (10).

On the other hand, breakthrough reactors with positive STs might be patients who experienced hypersensitivity reactions to the same ICM of *index* reaction despite premedication (in those cases in which ICM was unknown), or patients with multiple ICMs allergy due to cross-reactive compounds.

All these considerations reflect the heterogeneity of the breakthrough reactors' condition; suggesting that a considerable number of breakthrough reactors are probably patients who experience non-allergic hypersensitivity reactions or patients in which breakthrough reactions could be mostly prevented by a proper skin testing after *index* reaction. Our analysis is limited by the role of the *in vivo* tests in breakthrough reactions, thus not including the *in vitro* diagnostics (as basophil activation test) or triptase levels, which may have contributed to explain the results we achieved, especially for those patients who experienced an IR. Similarly, it would be useful to know if patients with a supposed non-clearly immune-mediated rash had a prior history of cutaneous manifestation as atopic dermatitis, pressure urticaria or dermagraphism, but these data are missing because of the retrospective nature of our analysis.

Unlike from patients with IR, the lower ST positive rate in breakthrough reactors with NIR is not easy to explain and we can't offer a possible explanation of the responsible mechanism. Unfortunately, we didn't use the drug provocative test (DTP) with an alternative ICM, which could help in identification of a safe, alternative compound, especially for patients with NIR (17-20), increasing the diagnostic yield. The usefulness of DTP in contrast media hypersensitivity is a recent acquisition and the procedure needs to be standardized (19).

Other limitations consist of as the sample size of the patients' cohort or the number of ICM tested. We performed the STs with iomeprol, iopromide, and iodixanol in all patients, since only these 3 ICM were used in our Institute in the last 8 years. A fourth ICM (iopamidol, 2 patients) was added for STs only for those patients who experienced the *index* reaction in other hospitals, with a known ICM different from the previous three. Although ENDA study group used at least four ICM for STs (10), our control group showed a rate of positive STs performed between 2 and 6 months comparable to that of ENDA study.

Finally, we performed STs using also 1:1 ICM dilution, which is not recommended by ENDA because of the risk of false positives (10), albeit several authors already used it with different results (11-14). Since in our experience 1:1 dilution of ICM may be useful if carefully read by the experienced allergist, we performed it in our cohort of patients. Furthermore, the STs rate of patients with prior breakthrough reaction was lower than the control group albeit 1:1 ICM dilutions, and STs rate of control group was not substantially higher compared to those reported by ENDA (10).

Despite these limitations and the heterogeneity of our cohort, we first observed that patients with prior breakthrough reactions have lower immunologically proven ICM reactions (with positive STs) compared to non-breakthrough reactions. Our results reappraise the role of breakthrough reactions; some of those are probably non-allergic hypersensitivity reactions or true allergic reactions that could be prevented by a proper, well-timed diagnostic skin testing. Larger prospective studies are needed to confirm these results, with a more careful analysis of patients' risk factors, a laboratory assessment that includes an *in vitro* allergy diagnostics, as for example tryptase levels during acute reaction for patients with IR, and hopefully DTP with an alternative ICM for selected cases, especially those with ST negative NIR.

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Omalizumab use during pregnancy for CIU: a tertiary care experience

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Key Words
urticaria; pregnancy; Omalizumab

Summary
The treatment of antihistamine and steroid resistant Chronic Idiopathic Urticaria (CIU) during pregnancy poses a challenge due to teratogenicity of immunosuppressants. Omalizumab is a recently FDA approved therapy for CIU and is classified as pregnancy category B. We present an initial series of subjects treated at a tertiary care center for antihistamine and steroid resistant CIU with omalizumab who became pregnant during therapy.

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Chronic Idiopathic Urticaria (CIU) affects 1% of the US population (1). There is a reported increase in prevalence of CIU among female groups with the highest birth rates in the US, those with a median age of 35 years (2). However, there is a lack of published data regarding prevalence of urticaria during pregnancy and its association with pregnancy complications and fetal outcomes. Previously, H1 antihistamines were the only approved therapy in the US for CIU and until today are considered first line therapy. However, nearly 50% of patients with CIU are unresponsive to antihistamine therapy alone. Corticosteroids are frequently incorporated in their management. Known pregnancy complications from steroid use include pre-eclampsia, gestational diabetes, primary cleft palate, neonatal adrenal insufficiency and low birth weight (3). Omalizumab, currently 4th line of therapy, is a pregnancy category B drug recently FDA approved for CIU.

We report a series of four female subjects, between the age of 25 and 28, treated with Omalizumab for antihistamine and steroid resistant urticaria, who became pregnant during therapy. Three of the four patients had a concomitant history of asthma demonstrated by pulmonary function tests, and two had a diagnosis of allergic rhinitis with positive skin testing. All four patients had failed multiple combination regimens that included high doses of first and second generation antihistamines coupled with H2 blockers and a leukotriene antagonist. Three patients were on immunosuppressive therapy with hydroxychloroquine, dapsone or cyclosporine without response. All subjects had received prednisone and two patients required chronic steroid therapy. All patients underwent workup including normal CBC, CMP, TSH and tryptase level. After failing previous regimens they were all started on Omalizumab at a dose of 300 mg subcutaneously (SC) every 28 days. Within the first month of therapy, all patients reported significant improvement of their symptoms demonstrated by lower urticaria index scores, decreased medical utilization and weaning of steroids. Three patients had been on

Omalizumab treatment for a year prior to pregnancy, one had been on treatment for only two months. All patients were informed on risks, benefits and previously reported outcomes of Omalizumab therapy for asthma during pregnancy (EXPECT) prior to proceeding with therapy. They were followed monthly, all patients had normal prenatal care, full term deliveries and no pregnancy or fetal complications.

To our knowledge, there are no published randomized controlled studies of Omalizumab in pregnancy. Reproductive studies on Cynomolgus monkeys at SC doses up to 10 times the maximum recommended human dose (75 mg/kg) failed to show harm to the fetus (4). Our reported experience is in agreement with previous reports of the EXPECT trial (5). Omalizumab remains a 4th line therapy for treatment of CIU but its excellent efficacy, symptom resolution and label as a pregnancy category B postulates it as an alternative option in pregnant patients that are unresponsive to antihistamines.

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Desensitization to Mycofenolate Mofetil: a novel 12 step protocol

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Key words
desensitization; IgE reactions; adverse drug reaction

Summary
The use of MMF has become standard practice in many solid organ transplant recipients due its efficacy and favorable risk profile compared to other immunosuppressants. There has been a single case report of successful MMF desensitization. However, this protocol did not follow current Drug practice parameters. We report a successful desensitization to MMF in a double heart-kidney transplant recipient

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Introduction

Mycophenolate mofetil (MMF) has largely replaced azathioprine as the preferred drug in organ transplant recipients, and more recently has also been used as a glucocorticoid-sparing agent for the treatment of several rheumatologic diseases. It selectively inhibits T- and B-lymphocyte proliferation by reversibly inhibiting the enzyme, inosine monophosphate dehydrogenase. This enzyme is crucial to the de-novo synthesis of guanine nucleotides by catalyzing the conversion of inosine monophosphate to guanosine monophosphate. Thus, by inhibiting the synthesis of purine nucleotides, it results in decreased B- and T-lymphocyte proliferation, and decreased antibody production (1).

MMF is usually tolerated well in most patients, with the most common side effects being gastrointestinal symptoms and leukopenia. Unlike hypersensitivity reactions, these adverse effects usually resolve with dose adjustments. Hypersensitivity to MMF is rare (2,3), with only two previous case reports in the literature (4,5). When a patient is suspected of having an IgE mediated hypersensitivity to MMF, and it remains the preferred drug over other immunosuppressants, then desensitization may be a safe alternative. Drug desensitization is the induction of a temporary state of tolerance (6). Drug tolerance is defined as a state in which a patient with a drug allergy will tolerate a drug without an adverse reaction. By inducing tolerance, it modifies an individual's response to a drug temporarily, and in so doing, allows safe treatment with that drug. Desensitization is indicated where an alternative, non-cross reacting medication cannot be used or is not equally efficacious. Induction of temporary tolerance can involve both IgE and non-IgE immune mechanisms, and even undefined mechanisms (7). Desensitization involves administering incremental doses of the drug over hours to days. The state of tolerance that results from desensitization is only maintained while the patient is taking the drug. Upon discontinuation of the drug, tolerance is lost within hours to days (7).

Case Report

We report a successful desensitization to MMF in a transplant recipient. A 46-year-old African-American female with a past medical history of systemic lupus erythematosus (SLE) induced dilated cardiomyopathy and end stage renal disease was scheduled for a double cardio-renal transplant at our institution.

About 10 years prior to her planned transplant, she was placed on MMF, prednisone and cyclophosphamide during an acute flare of her underlying lupus. After a few days she started to experience pruritus of her lips several minutes after ingestion of MMF. After being on MMF for two weeks, she developed facial hives, pruritus and angioedema within 12 hours of her last dose. She was then advised to stop taking MMF and subsequently noticed complete resolution of her symptoms within 3 days while continuing on prednisone and cyclophosphamide.

Due to her history being concerning for an IgE mediated reaction to MMF, her transplant team consulted our Allergy and Immunology Department to consider a rapid drug desensitization procedure. We reviewed the literature and found two previous case reports (4,5). Upon review, both protocols utilized on the published case reports (4,5), deviated from current drug allergy practice parameters set forth by Solensky et al (7). One of the most noticeable deviations was that the protocol previously developed (4), involved giving incremental doses of MMF over 3 days, instead of several hours.

Table 1 *- Mycophenolate Mofetil Oral Desensitization.*

Step	Time (H:MM)	Dose (mg)	Oral volume (200 mg/ml1)
1	0:00	0.25	0.00125
2	0:20	0.5	0.0025
3	0:40	1	0.005
4	1:00	2	0.01
5	1:20	4	0.02
6	1:40	5	0.025
7	2:00	16	0.08
8	2:20	32	0.16
9	2:40	64	0.32
10	3:00	125	0.625
11	3:20	250	1.25
12	3:40	500	2.5
Total	3:40	999.75	4.99875

[1]Mycophenolate solution was prepared via diluting a stock solution of mycophenolate (200 mg/ml) with Ora-Plusâ until a 4 mg/ml solution was obtained.

In our patient, skin testing to MMF was planned to gain further insight on reactivity and, however the patient had a blunted response to the histamine control. Given the urgency of the situation and inability to interpret our skin tests, we decided to proceed directly with a desensitization procedure. We designed a novel 12-step desensitization protocol (**table 1**), in accordance with the current drug allergy practice parameters recommendations.

Following her back-to-back cardio-renal transplant, the patient was placed on azathioprine, tacrolimus and methylprednisolone, pending her desensitization to MMF.

In an intensive care unit setting, an oral desensitization with MMF was performed with diphenhydramine 25 mg IV and famotidine 20 mg IV given as premedication. Incremental doses were given every twenty minutes, reaching a target dose of 500 mg (cumulative 1000 mg) without adverse reactions. She was then successfully continued on the target dose of MMF 500 mg twice daily and tolerated it well. It was subsequently discontinued on a future hospitalization, four months later, due to concern that it may have contributed to an incidental finding of leucopenia. She has since been placed on azathioprine and was doing clinically well at her last follow-up, 12 months after her transplant.

Conclusion

The use of MMF has become standard practice in many solid organ transplant recipients due its efficacy and favorable risk profile compared to other immunosuppressants. Although an IgE mediated allergy to MMF is rare, it may be increasingly encountered due to its increasing use. Our protocol can be applied to other such patients to achieve a successful desensitization.

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An unexpected cause of anaphylaxis: potato

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asthma; anaphylaxis; atopic dermatitis; childhood; potato

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Summary
Immediate reactions against contact to raw potato has been reported in adults with generally being in the form of an oral contact dermatitis or contact urticaria, but it may also manifest as rhinitis symptoms, wheezing or even anaphylaxis. Cooked or raw potato allergy has been rarely reported in children as some is being immediate and others being late reactions, and it usually results from ingestion. Herein, we report two cases with a background of allergic diseases developed anaphylaxis one with cooked potato and the other one with raw potato.

Introduction

It is estimated that 5% of young children are affected by food allergy with increasing prevalence. Food-induced allergic reactions account from diversity of symptoms and disorders including the skin, gastrointestinal and respiratory tracts which can be ascribed to IgE-mediated and non-IgE-mediated mechanisms (1). Foods have a pathogenic role in a subset of children with atopic dermatitis (AD) and asthma (1). Allergy to cow's milk, eggs, and cereal is more widespread in atopic infants and younger children (2). However, it is considered that allergy to potato is uncommon in contrast to above-mentioned foods. As in Western countries, white potato *(Solanum tuberosum)* is a very common ingredient in the diet of Turkey. Its cooked form is introduced in the child's diet generally around the age of 4 to 6 months as one of the first solids foods (3). In children, allergy to cooked form has been reported, including both immediate and late severe reactions, and even with anaphylaxis (3,4). In the literature, allergic reaction against to raw form has also been reported in the children (5,6). Here, we presented two cases with anaphylaxis against cooked and raw potato.

Case report

Case 1

An 11-months old boy presented to our clinic with flushing and swelling at cheeks and lips, ocular itching and erythema, nasal itching, sneezing and cough. In his history, it was found that raw potato was given to alleviate discomfort during eruption; followed by allergic reaction against raw potato. It was also seen that the parents described presence of atopic derma-

titis lesions since he was 2 months old. No reaction was expressed when fed by cooked potato and/or after maternal potato ingestion or feeding by foods containing potato. No atopia or allergic disorder was present in the family history. In physical examination, eczematous lesions were observed at cheeks, flexural sides of upper and lower extremities. No abnormal finding was observed in systemic examination. Laboratory test revealed absolute eosinophil count of 270/mm^3, percent eosinophil of 4.5%, total IgE of 38 IU and serum potato specific IgE 4.92 kU/L. We applied a test panel with aeroallergens and food allergens including: Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria alternata, cow's milk, walnuts, hazelnuts, peanuts, sesame seeds, wheat, egg whites, tuna fish, soybean bean and histamine (10 mg/ml of histamine phosphate) as positive and 0.9% sterile saline as negative controls. Standardized extracts (Stallergenes; Antony, France) were used, and SPTs were evaluated 15 min after application and were considered positive if the mean wheal diameter was ≥ 3 mm compared with the negative control. As a result, we found SPT positivity against walnut 6 x 6 mm and egg white 8 x 7 mm. In prick-to-prick test using raw potato, the patient was found to be sensitive against raw potato 10 x 12 mm, histamine 5 x 6 mm (**figure 1**). In the provocation test using potato, flushing and induration was detected after contact of raw potato to lips (**figure 2**). Egg, walnut and raw potato was eliminated from his diet. No latex allergy was detected. The family was counseled about potential allergic disorders such as pollen allergy, allergic rhinitis and asthma.

Case 2

A 3-years old boy presented to our clinic with cough, wheezing and dyspnea over 5-10 days of each month within previous year. It was found that the patient presented to emergency department in all episodes and received inhaler salbutamol therapy during these episodes. The parents described cough, abdominal pain and vomiting were developed after consumption of cooked potato for the first when he was one year old. It was found out that father and grandfather had asthma. No abnormal finding was detected in the physical examination. Laboratory test revealed absolute eosinophil count of 540/mm^3, percent eosinophil of 4.5%, total IgE 80 IU and serum potato specific IgE 125 kU/L. Skin testing was done with a standard test panel for aeroallergens (7) and food allergens including: Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat, dog, Alternaria alternata, Cladosporium herbarum, Cynodon dactylon, grassmix, treesmix, composite, cockroach and cow's milk, walnuts, hazelnuts, peanuts, sesame seeds, wheat, egg whites, tuna fish and soybean. No sensitivity to inhaler and food allergens was detected in the skin prick test. Skin testing to fresh and cooked potato by prick to prick method was found to be markedly positive respectively 10 x 12 mm and 8 x 7 mm (**figure 3**).

Figure 1 *- Positive results to raw potato (3) and positive control (histamine, 2) in prick by prick. The tests with latex (4) and physiologic saline (1) are negative.*

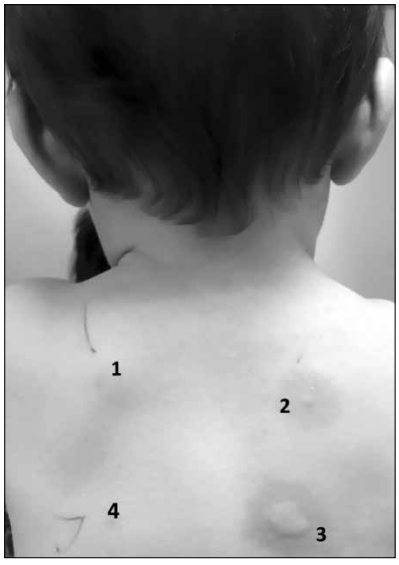


Figure 2 *- Positive raw potato challenge (labial and face edema, erythema).*

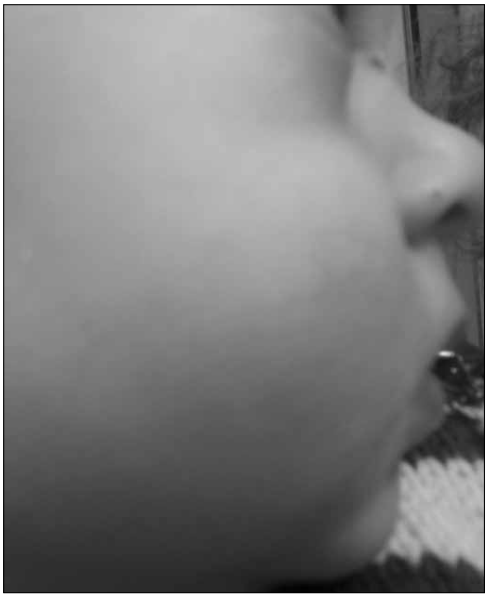


Figure 3 *- Positive results to raw potato (4), cooked potato (3) and positive control (histamine, 2) in prick by prick. The tests with latex (5) and physiologic saline (1) are negative.*

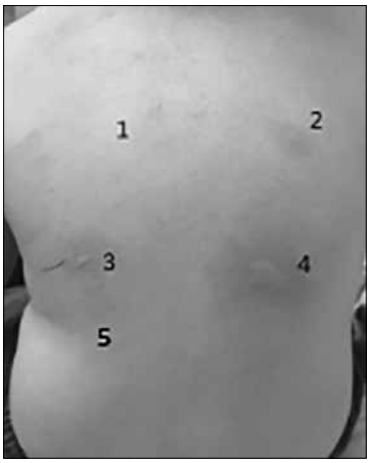
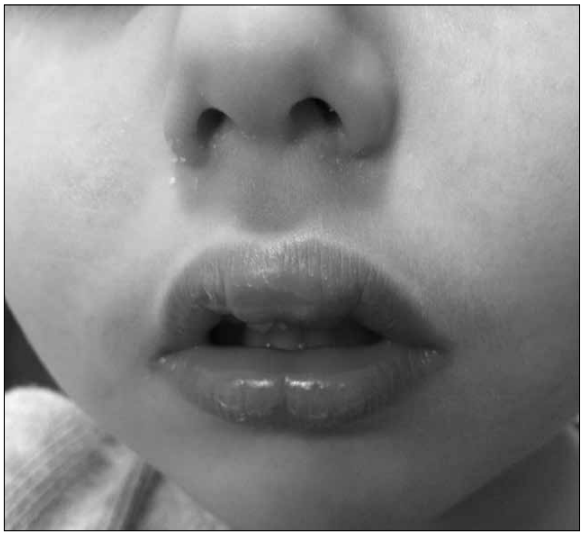


Figure 4 *- Positive raw potato challenge (labial edema, erythema and induration).*



In the provocation test using raw potato, flushing and induration was detected after contact of raw potato to lips (**figure 4**). In the first step of provocation test using cooked potato, nausea and mild abdominal pain were observed and the test was discontinued as the parents declined to continue. No latex sensitivity was detected. Potato was eliminated from his diet. Asthma therapy was prescribed. Regular follow-up was scheduled for potential pollen allergy.

Discussion

The vast majority of anaphylaxis cases in children are related with food, especially cow milk and eggs. Although potato is widely consumed in our region as in Europe, adverse reactions to potato are unusual. Both cooked and raw form of potato can cause allergies. In adults, allergy to raw potato is generally considered as a manifestation of oral allergy syndrome in patients with pollen allergy. It is particularly observed in housewives, who experience itching, rhinoconjunctivitis, and, in some cases, asthma or even anaphylaxis during the peeling of potatoes (8). However, allergy to cooked potato have only been reported in children so far and it has been reported that allergy to cooked potato may involve both immediate and late severe reactions, and even anaphylaxis (3,4,9,10). Potato related anaphylaxis is rare. Monti et al. (11) reported an 8-month-old patient developing anaphylaxis with cooked potato, while Beausole et al. (12) described a 4 year old patient developing anaphylaxis with raw potato. In the study by De Swert et al. (3) 36 cases with potato allergy were evaluated, three of which were admitted with clinical features of anaphylaxis. Symptoms of anaphylaxis were observed after contact to raw and cooked potato in our cases.

In a previous study declareted that AD was the most common clinical feature present with potato allergy (3,9,13). De Swert et al. (5) evaluated children with potato allergy, all patients had atopic dermatitis. In another study by De Swert et al. (10) it was found that all subjects apart from one with potato allergy had eczema. In the study by Majamaa et al. (4) in which skin testing, oral challenge responses to potato and the occurrence of immunoglobulin E antibodies to patatin (Sol t 1) were evaluated in infants, it was found that all patients had atopic dermatitis. In another study, it was reported that there was AD in 33 of 40 patients.

Respiratory symptoms (wheeze / rhinitis) were the second most common symptom. Foods rarely cause respiratory symptoms. In the study by De Swert et al. (3) it was found that there was wheezing / rhinitis in 40% of those patients with potato allergy. A case report by Quirce et al. (8) reported two housewives in whom asthma findings developed after handling raw potato.

Thus far, five potato allergens have been defined; the glycoprotein "patatin" (Sol-t-1) is the most important of these that shows a significant homology with a latex allergen, leading to the possibility of cross-reaction (4,14,15). Patatin is considered to be a heat-labile allergen. In addition, 4 IgE-binding potato proteins (cathepsin D-, cysteine-, and aspartic protease inhibitors) were identified and designated as Sol t 2, Sol t 3.0101, Sol t 3.0102, and Sol t 4, belonging to the family of soybean trypsin inhibitors (Kunitz type) by Seppälä et al. (14). Although patatin is considered to be a heat-labile allergen, it has been shown that its IgE interaction is strongly influenced by other potato proteins in terms of heat lability (16). The development of symptoms in

some patients with only raw potato or unprocessed potato or after oral intake, can be attributed to heat-labile potato proteins, which are unstable in the presence of digestive enzymes and gastric acid or lose their allergenic properties after cooking (3). Reaction with raw potato is observed in the presence of a reaction against patatin usually in the form of erythema and urticaria.

There are different allergens expressing cross-reaction with potato. Potato is one of the foods implicated in the latex-fruit syndrome, and it has been questioned whether latex sensitization precedes or follows the onset of food allergy (14,15). Latex sensitivity was not detected in our patient. Others important allergens include birch pollen and grass pollen. In our patient, follow-up was scheduled for the development of seasonal allergic rhinitis and pollen sensitivity.

Here, we aimed to emphasize potato allergy, a rare entity, and to remind potential disorders that could develop with or after potato allergy.

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Heavy metal and tattoo: an allergy and legislative problem

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Key words
tattoo; heavy metal; copper; allergy

Summary
We presented an interesting clinical case of a 23 years old man presented with a 2-week history of pruritus, erythema and papules on legs, arms and trunk. These lesions developed 2 months after tattooing. It showed positive patch test reaction to Copper and Disperse Blu.

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It is estimated that more than 24% of American adults have one or more tattoos, and the practice is gaining social acceptability and is becoming more popular also in Italy, especially among adolescents (1).

In the last few years, the demand for new colors has increased. People demand for brighter colors and different shades. Pigments have been enriched with components to increase the brightness, like azopigment and heavy metal for new shades.

Heavy metal toxicity has proven to be a major threat, and there are several health risks associated with it. The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body, while at certain times they may even interfere with metabolic processes. Some metals get accumulated in the body and food chain, exhibiting a chronic nature. Various public health measures have been undertaken to control, prevent and treat metal toxicity occurring at various levels, such as occupational exposure, accidents and environmental factors. Metal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation (2).

As regards tattoo pigment, the legislation is not clear. In Italy there are different regional legislations.

Copper (Cu) is a vital mineral essential for many biological processes. The vast majority of all Cu in healthy humans is associated with enzyme prosthetic groups or bound to proteins. Cu homeostasis is tightly regulated through a complex system of Cu transporters and chaperone proteins. Excess or toxicity of Cu, which is associated with the pathogenesis of hepatic disorder, neurodegenerative changes and other disease conditions, can occur when Cu homeostasis is disrupted. The capacity to initiate

oxidative damage is most commonly attributed to Cu-induced cellular toxicity. Recently, altered cellular events, including lipid metabolism, gene expression, alpha-synuclein aggregation, activation of acidic sphingomyelinase and release of ceramide, and temporal and spatial distribution of Cu in hepatocytes, as well as Cu-protein interaction in the nerve system, have been suggested to play a role in Cu toxicity (3).

Cu occurring also in cosmetics may undergo retention, and act directly in the skin or be absorbed through the skin into the blood, accumulate in the body and exert toxic effects in various organs. Some cases of topical (mainly allergic contact dermatitis) and systemic effects owing to exposure to metals present in cosmetics have been reported.

We presented a clinical case of a 23 years old man presented to our department with a 2-week history of pruritus, erythema and papules on legs, arms and trunk. These lesions developed 2 months after tattooing (**figure 1**). General physical examination was normal.

The patch test was performed using the standard series SIDAPA. It resulted negative. So, we decided to execute the special series F.I.R.M.A. for tattoo (copper sulphate 1% water, dimetilaminoazobenzene-p 1%, aminoazotoluene-o 1%, blue scattered 3 1%, blue scattered 124 1%, yellow scattered 3 1%, orange scattered 3 1%, red scattered 1 1%, gentian violet 2%, cadmium chloride 1% in water, nickel sulphate 5%, iron chloride 2% in water, potassium dichromate 0.5%, chromium trichloride 2%, aminoazobenzene-p 0.25%, cobalt chloride 1%, aluminum chloride 2%, titanium dioxide 0.1%, zinc 2.5%, mercury chloride 0.05% in water, kathon cg 0.01% in water, phenol 0.5%, ethylenediamine hydrochloride1%, phenylenediamine base-p 1%, formaldehyde 1% in water, phthalic anhydride 1%, rosin 20%, dibutyl phthalate 5%, hexamethylenetetramine 1%, benzophenone 5%).

Our patient showed positive patch test reaction to Copper (++ positive) and Disperse Blu (+ positive) (**figure 2**).

We proposed to our patient to remove the tattoo with laser therapy, but he refused it. So, we performed local infiltration of cortisone for four weeks, with temporary resolution of clinical manifestation.

The tattoo phenomenon is expanding rapidly, and involves mainly young people between 16 and 25 years. Great attention must be put to the pigments used. There are new substances, often little known, and allergic reactions to these pigments are increasing rapidly. Deciding to inject a pigment on your skin deserves great attention, even more so choosing the pigments to be used. We recommend that you always perform a patch test before getting a tattoo. (4,5).

Figure 1

Figure 2

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Allergy in urban elderly population living in Campania region (Southern Italy). A multicenter study

On behalf of Italian Association of Hospital and Territorial Allergologists (AAIITO - Campania Region)

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Key words

allergy; allergic rhinitis; allergic sensitization; bronchial asthma; Campania region; elderly; hypersensitivity

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Summary

Given the increasing life expectancy observed in Western countries, there is a marked interest to know more about how aging could influence respiratory health. The aim of our study was to assess the prevalence, clinical characteristics and age of onset of allergic sensitization and clinical symptoms in a sample of atopic elders living in Campania region area (Southern Italy). Fourteen Allergy units or Centres examined a total of 462 patients. In this context 215 (46.53%) had positive skin prick tests (SPTs) to at least one allergen and were diagnosed with respiratory allergy. Parietaria *represents the most common sensitizing agent in elders living in Campania region, followed by dust mites, grass pollen and* Olea europaea. *A relatively high percentage of atopic subjects suffered from respiratory symptoms at a fairly advanced age, namely 8.3% at 60-64 years, 10.2% at 65-70 and 5.7% at > 70 years. In conclusion, the prevalence and clinical significance of airway allergic sensitization in the elderly living in Campania region is more significant than expected in latter stages of life. Physicians should not neglect the role of atopy as a risk factor for the onset of allergic respiratory symptoms even in elderly patients.*

To the Editor

Given the increasing life expectancy observed in most Western countries, there is a marked interest to know more about how aging could influence respiratory health. Aging influences not only the respiratory function but also the immune response to infectious agents and the environment (allergens and air pollutants) (1).

It has been shown that asthma and allergic diseases are not uncommon in the elderly, and the prevalence of asthma appears to be increasing over the past decades (2). Although allergens and the allergic sensitivity have a lesser impact compared to younger populations, allergy remains a relevant problem in the elderly (3). Furthermore, multi-morbidity is certainly the most important problem related to old age, being associated with disability, institutionalization, poorer quality of life and higher frequency of adverse events related to multiple concomitant treatments, and ultimately death (4). Other aspects on the management of respiratory allergic disorders in the elderly compared to other age groups were also described (5-19). Since Campania region is inhabited by the youngest population in Italy, studies on airway allergic sensitization have been carried out mainly in children and adults.

The aim of our study was to assess the prevalence, clinical characteristics and age of onset of allergic sensitization and clinical symptoms in a sample of atopic elderly subjects living in the Campania region (Southern Italy).

Fourteen Allergy units or Centres belonging to the Italian Association of Hospital and Territorial Allergologists (AAITO - Campania region), uniformly distributed over the whole territory of Campania region (13.595 Km^2, 6.074.882 inhabitants) participated in this cross-sectional study. The same protocol was shared by all participating centers; each Centre collected the results of allergy consultations of consecutive outpatients, aged > 60 years, referred for suspected or current respiratory allergy (asthma and/or rhinitis). Patient enrollment started on January 1 and ended on June 30, 2014.

A case report form (CRF) specifically designed for this study was completed during the screening consultation of each patient. The standardized form reported: demographic data, type and duration of respiratory symptoms, pet ownership, results of the skin prick tests (SPTs), age of onset of respiratory symptoms. The diagnosis of respiratory allergy has been carried out according to the International Guidelines (20,21).

The commercial allergen extracts used for screening SPTs were provided by ALK-Abellò Group (Milan, Italy). A standard panel of allergens was used, including *Dermatophagoides pteronyssinus* and *D. farinae, Alternaria alternata, Cladosporium herbarum,* cat and dog dander, *Parietaria,* Grass pollen mix, *Artemisia vulgaris, Olea europaea, Betula pendula, Cupressus sempervirens* and *Corylus avellana.* This allergen panel covers the main causative agents of respiratory allergy in Campania region. Positive (10 mg/ml histamine HCl) and negative (saline solution in glycerine-phenol solution) controls were used. SPTs were performed and interpreted according to International Guidelines (22); results were read after 15 minutes and expressed as the mean of the major wheal diameter plus its orthogonal. A skin reaction of 3 mm or greater was considered positive. Wheal profiles were outlined using a fine-point marking pen and transferred by adhesive tape onto the patient's form.

Patients with chronic infectious diseases, malignancies or dysmetabolic diseases, severe cutaneous disorders, negative skin reaction to histamine, or undergoing treatment with drugs interfering with skin response were excluded from the study (23,24).

A total of 462 patients were examined (females 291, 62.9%; males 171, 37.01%). Two hundred and fifteen subjects (46.53%) had positive SPTs to at least one allergen and were diagnosed with respiratory allergy, the remaining 247 (53.46%) were SPTs-negative. Female sex was predominant either in atopic (females 135, 29.2%; males 80, 17.3%) and non-atopic elders (females 156, 33.8%; males 91, 19.7%). *Parietaria* represents the most common sensitizing agent in elderly allergic patients living in Campania region, followed by dust mites, grass pollen and *Olea europaea* (**figure 1**), irrespective of age of symptom onset (< 50 or > 50 years) (**figure 2**). This data differs from previous reports on children and adults living in Campania region and Naples area, where the most common sensitizing agents were dust mites followed by *Parietaria*, Grass pollen and *Olea europaea* (25-28).

Although a significant proportion of allergic elders reported the first onset of respiratory symptoms under 40 years, a high proportion of atopic subjects suffered from respiratory symptoms first occurring in late adulthood namely 8.3% at 60-64 years, 10.2% at 65-70 years and 5.7% > 70 years (**figure 3**). More than a half of these elders experienced a late onset respiratory allergy, showing that late sensitization occurs more frequently than previously thought.

Only a small percentage of these subjects suffered from allergic respiratory symptoms during adolescence. As expected, individuals with non-allergic respiratory symptoms are less commonly seen in younger age compared to older age (**figure 3**).

In conclusion, our data show that the prevalence and clinical importance of airway allergic sensitization in the elderly living in Campania region is more significant than expected, especially in late adulthood. This finding should be taken into account by clinicians and allergologists, who should not neglect the role of atopy as a risk factor for the onset of allergic respiratory symptoms even in the elderly.

Authorship

All authors contributed equally in the writing and revision of the manuscript.

Figure 1 *- Percentages of all elderly patients sensitized to common aero-allergens.*

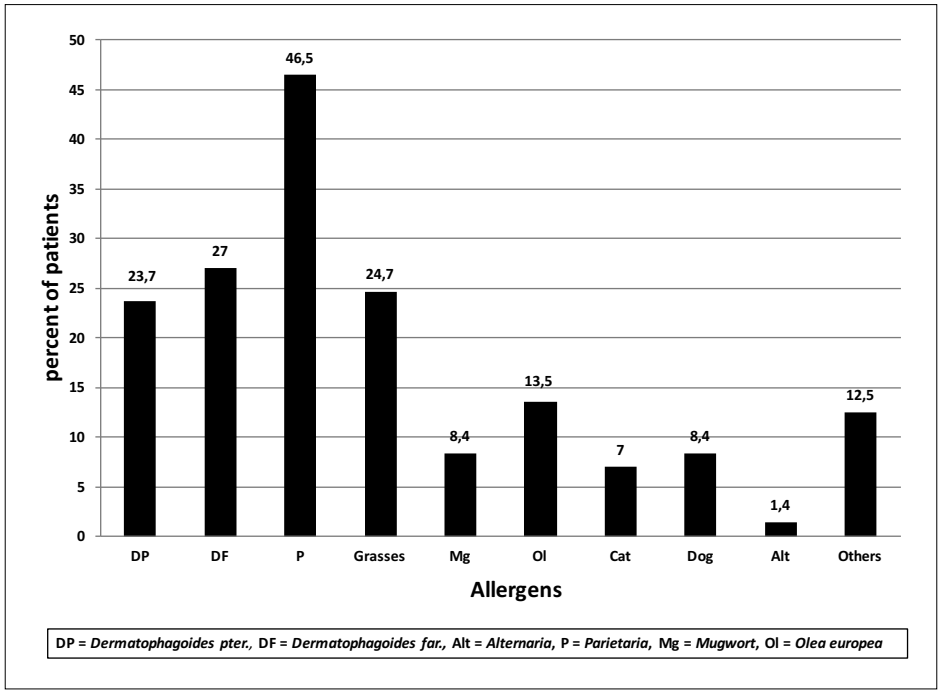


Figure 2 *- Percentages of elderly patients sensitized to common aero-allergens according to the time of onset of symptoms (before or after 50 years of age).*

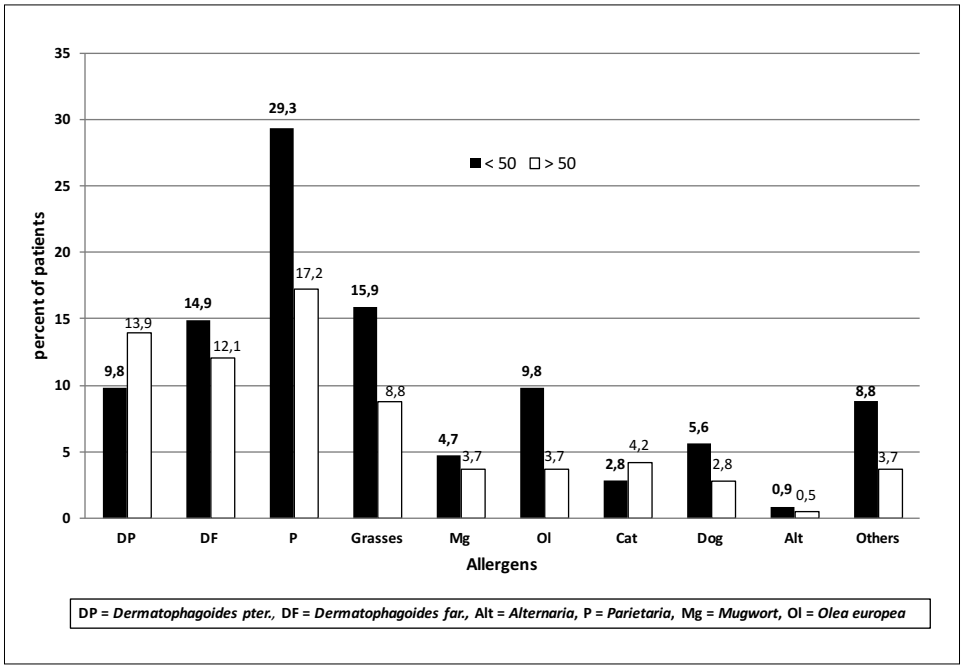
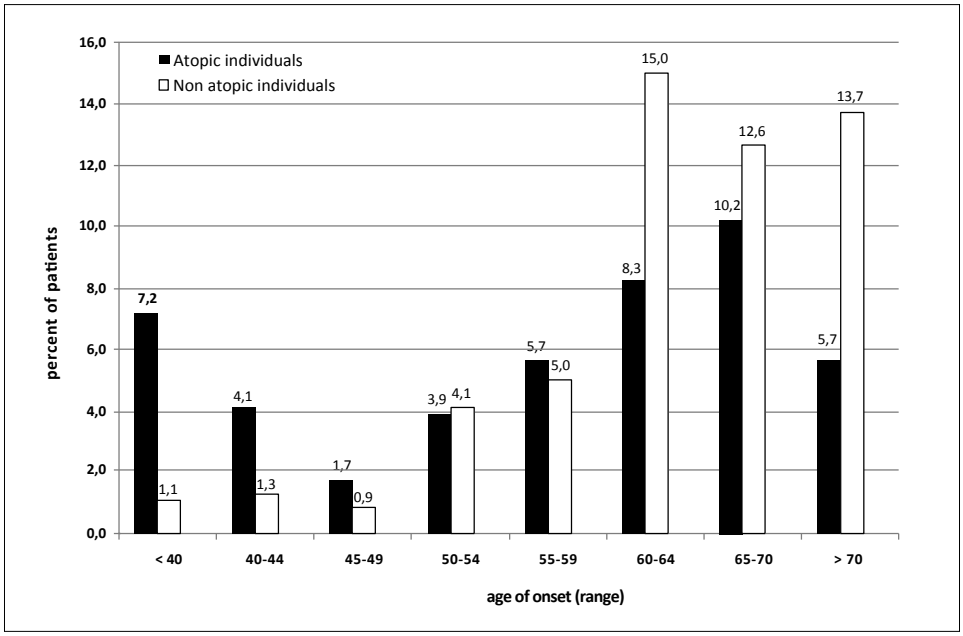


Figure 3 *- Age of onset of respiratory symptoms in atopic / non atopic elderly patients.*



Conflict of interest and financial resources

All authors declare that they have no conflict of interest and that the study has been carried out without any financial support.

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