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Anaphylaxis

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Adrenaline; anaphylaxis; idiopathic anaphylaxis; biomarkers; co-factors; endotypes; exercise-induced anaphylaxis; management; phenotypes; preventive measures; risk factors.

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Summary

Anaphylaxis is the most severe systemic hypersensitivity reaction, and it can be life-threatening or even fatal. It involves the activation of multiple immune and non immune pathways beyond IgE, thus exhibiting different phenotypes. New symptoms of hypersensitivity caused by chemotherapy drugs, monoclonal antibodies, and biological agents have been suggested to be recognized as anaphylaxis phenotypes. No biomarker has been described that allows an unequivocal diagnosis of anaphylaxis. Moreover, more biomarkers for specific endotypes are needed to stratify severity, to predict risk, and to optimaze tretament choice in the individual patient.

Food, drugs and stinging insects represent the most commly identified triggers. Idiopathic anaphylaxis is a diagnosis of exclusion and it can hide a clonal mast cell disorder.

Individual risk factors and co-factors may influence the severity of anaphylaxis or its onset, and they should be identified to implement the appropriate measures to prevent recurrence.

Prompt recognition and treatment are critical in anaphylaxis, adrenaline being the first-line saving therapy. Individualized anaphylaxis action plan should include avoidance measures, prescription of an adrenaline autoinjector, education, optimal management of relevant comorbidities, venom specific immunotherapy, food oral immunotherapy, and drug desensitization, when appropriate.

However, the quality of acute and long-term anaphylaxis management is variable influencing the poor outcomes experienced by many patients. Clinical practice guidelines have the potential to improve outcomes, but they often prove challenging to implement in routine clinical care.

Introduction

Anaphylaxis is the most severe systemic hypersensitivity reaction, it involves multiple organ systems, can be caused by a number of triggers and conditions, and be deadly. A number of slightly different definitions for anaphylaxis has been used in different Guidelines (1-6). In these guidelines, the independently developed definitions of anaphylaxis for clinical use all include the concepts of a serious, generalized or systemic, allergic or hypersensitivity reaction that can be life-threatening or fatal. Importantly, none of the definitions include the word "shock" (1-3). The correct term "anaphylaxis" is preferred to "anaphylactic shock" because shock is not necessarily present in patients with anaphylaxis and it is used in preference to terms such as "anaphylactoid reaction", or "pseudo-anaphylaxis" (1-3). All Guidelines report that anaphylaxis is a "life-threatening reaction", even though in general mortality or morbidity do not seem to have increased in recent decades (7-9), despite the vast majority of anaphylaxis reactions are not treated properly with prompt administration of adrenaline. However, as it is not possible to predict the severity of reaction and early adrenaline administration may reduce the risk, the concept of "life-threatening" must be present in the anaphylaxis definition (10).

The anaphylaxis guidelines of the World Allergy Organization (WAO) (2) and of the European Academy of Allergy and Clinical Immunology (EAACI) (1) have established 3 sets of clinical crite-

ria for the diagnosis of anaphylaxis, confirming the proposal of the second symposium on the definition and management of anaphylaxis summary report - Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium (4). In short, anaphylaxis is highly likely in the case of an acute onset of an illness (minutes to several hours) affecting at least 2 different organs (*e.g.* skin-mucosal tissue, airways, gastrointestinal apparatus, cardiovascular system) or in the case of a a reduced blood pressure after exposure to known allergen for that patient (minutes to several hours). Because anaphylaxis mimics common syndromes, such as asthma and urticaria, and it can present without hypotension, its diagnosis is often missed or delayed.

For a number of good clinical reasons, very recently the Anaphylaxis Committee of the WAO proposed to revisit the definition and clinical criteria of anaphylaxis, in order to better capture the reality of anaphylaxis, simplify diagnosis, thus inproving the management (10). This proposal will certainly be the subject of worldwide discussion. It will probably has to take into account that there is a new understanding that atypical symptoms, such as pain, chills, fever can be seen during chemotherapy-induced anaphylaxis characterized by hypotension, desaturation, and cardiovascular collapse, which can lead to a new classification of anaphylaxis pathways (11). Moreover, clonal disorders, such as monoclonal mast cell activation syndrome, are considered part of the wide spectrum of anaphylaxis (11).

A minority of patients exhibit biphasic allergic reactions induced by a variety of causes,, in which signs and symptoms of anaphylaxis recur hours after initial resolution of anaphylaxis without re-exposure to the trigger (12). In addition to the biphasic reactions, patients who have IgE reactive with the oligosaccharide galactose-alpha-1,3-galactose, which is present in mammalian meat and in some therapeutic antibodies, can exhibit anaphylaxis after a delay of several hours during which no signs or symptoms are present (13).

Several classifications were proposed to assess the degree of severity of anaphylaxis; the most used in clinical practice is Ring's (14). New proposed severity scores from Brown and EAACI guidelines suggest simpler criteria, namely dividing reactions in mild, moderate or severe, or in grades according to local (grade 1) or systemic involvement (grade 2, 3), respectively (15, 16). In the latter, however, such proposed grading might be confusing for Hymenoptera venom allergy, given that local reactions are referred to local cutaneous involvement, rather than generalized urticaria.

Epidemiology and triggers

There is some evidence that the incidence of anaphylaxis may be increasing, but this may be due to changing clinical definitions or thresholds for presentation or admission.

A systemic review of the epidemiology of anaphylaxis in Europe (17) found the incidence rates for all-cause anaphylaxis ranging from 1.5 to 7.9 per 100,000 person-years. These data indicated that an estimated 0.3% (95% CI 0.1-0.5) of the population experience anaphylaxis at some point in their lives, with food, drugs, stinging insects, and latex being the most commonly identified triggers. Overall, the case fatality ratio from anaphylaxis was low, estimated at under 0.0001% (17).

A review by a Working Group of the American College of Allergy, Asthma, and Immunology including a number of non-European studies, concluded that the overall incidence of anaphylaxis was between 30-60 cases per 100,000 person-years and 950 cases per 100,000 person/years, with a lifetime prevalence 0.05-2.0% (18). The lower estimates by Panesar *et al.* may reflect differences in diagnostic criteria for anaphylaxis between Europe and North America.

Moreover, it is estimated that 1 in every 3,000 inpatients in US hospitals suffer from an anaphylactic reaction with a risk of death around 1%, accounting for 500 to 1000 deaths annually in this country (19).

Based on these statistics, anaphylaxis would fit well the definition of a rare disease, although it is not currently listed in rare diseases registries (20). In public health terms, anaphylaxis is considered to be an uncommon cause of death (2, 21-23). The case fatality rate is difficult to ascertain with accuracy. Accurate anaphylaxis mortality data are hampered by the limited recognition of this condition among health professionals, the absence of historical details from eyewitnesses, incomplete death scene investigations, paucity of specific pathologic findings at postmortem examination, and the under-notification of anaphylaxis, particularly in the International Classification of Diseases, Injuries and Cause of Death (ICD) (2, 20, 24). Although currently misclassified in the ICD, anaphylaxis is now one of the principal headings in the "Allergic and hypersensitivity conditions" section recently compiled for the forthcoming 11th Revision of ICD (ICD-11). Thanks to this inclusion, it is expected that anaphylaxis should be a public health priority and that it should therefore be formally added into the list of rare diseases in order to support awareness and quality clinical management of patients.

As for the triggers, a pan-European registry for severe allergic reactions collecting 3333 cases of anaphylaxis, showed that allergic reactions were mainly caused by food and insect venom and less often by drugs (25). Most reactions occurred within 30 min of exposure (80.5%); a delay of 4+ hours was mainly seen in drug anaphylaxis (6.7%). Symptom patterns differed by elicitor, with the skin being affected most often (84.1%). Usually previous milder reaction to the same allergen was reported by 34.2% (25).

Data collected in the European Anaphylaxis Registry from 2007 to 2015 (25), allowed to characterize anaphylaxis in children and adolescents (26). Food items were the most frequent trigger (66%), followed by insect venom (19%). Cow's milk and hen's egg were prevalent elicitors in the first 2 years, hazelnut and cashew in preschoolaged children, and peanut at all ages.

There was a continuous shift from food- to insect venom- and drug-induced anaphylaxis up to age 10 years. Vomiting and cough were prevalent symptoms in the first decade of life, and subjective symptoms (nausea, throat tightness, and dizziness) were prevalent later in life. Most incidents occurred in private homes (46%) and outdoors (19%). One third of the patients had experienced anaphylaxis previously (26).

Looking at epidemiological data from from intensive care units pediatric anaphylaxis admissions, 1989 patients were reported from 2010 to 2015 in the United States and Canada, the most common identified trigger being food (mainly peanuts) (27). One percent of patients died because of critical anaphylaxis, and identified triggers for fatal cases were food (peanuts and milk) and blood products (27).

Comparing European patients aged > 65 (elderly: 1, 123) with adults (18-64 years: 5.768) regarding elicitors, symptoms, comorbidities, and treatment measures, insect venoms were the most frequent elicitor in the group ($p < 0.001$), followed by drugs like analgesics and antibiotics (28). Food allergens elicited less frequently anaphylaxis ($p < 0.001$). Skin symptoms occurred less frequently in elderly patients (77%, $p < 0.001$). The clinical symptoms were more severe in the elderly (51% experiencing grade III/IV reactions), in particular when skin symptoms ($p < 0.001$) were absent. Most strikingly, a loss of consciousness (33%, $p < 0.001$) and preexisting cardiovascular comorbidity (59%, $p < 0.001$) were more prevalent in the elderly (28).

Risk factors and co-factors

The risk to develop severe reactions like anaphylaxis, may depend on several factors, including the allergens an individual patient is sensitized to, the degree of sensitization, the quality of binding allergens, probably also the relative proportions of antigen-specific immunoglobulin subtypes, the route of allergen application, and finally, the presence and 'amount' of risk factors and cofactors (29). According to some authors, 'risk factor' is a general term covering any factor, which may lead to more severe allergic reactions, including augmenting factors (also called aggravating factors), concomitant diseases and cofactors (30), while others distinguish between risk factors and co-factors (2, 31).

Very recently, processing the data from the European Anaphylaxis Registry (122 centers in 11 European countries) higher age (not related to concomitant cardiovascular or other diseases) and concomitant mastocytosis have been identified as the most important predictors for an increased risk of severe anaphylaxis (32). Vigorous physical exercise, male sex, and psychological burden were more often associated with severe reactions. Moreover, intake of beta-blockers and ACE inhibitor (ACE-I) in temporal proximity to allergen increased the risk to develop severe anaphylaxis exposition in logistic regression analysis, while ASA and AT-2 did not (32). Indeed, it was recently shown that beta-blockers (BBs) and the ACE inhibitor (ACEI) ramipril can directly promote mast cell activation and are associated with increased odds for severe anaphylaxis (33).

In contrast, a systematic review and metaanalysis of studies that assessed the influence of BBs and ACEIs on anaphylaxis showed low quality of evidence that the use of BBs and ACEI increases the severity of anaphylaxis, due to differences in the control of confounders arising from the concomitant presence of cardiovascular diseases (34).

In a large observational cohort study performed in the United States from 2005 to 2014, age of 65 years or older, medication as a trigger, and presence of comorbid conditions (specifically cardiac and lung disease) were associated with significantly higher odds of severe anaphylaxis (35). On the other hand, evidence showing that respiratory disease increases the severity of anaphylaxis according to a recent a systematic review and metaanalysis is low to moderate, although studies do not usually assess the importance of severity of asthma (36).

A genetic diversity should be also included among the host factors influencing anaphylaxis in some cases of food and drug allergy (37, 38). Polymorphisms affecting metabolism of mediators of anaphylaxis also can influence anaphylaxis severity, since PAF-AH activity levels inversely correlated with severity of anaphylaxis (39, 40), and subjects with variants in angiotensinogen were reported to have increased rates of Hymenoptera venom allergy (41). D816V mutations are found in some patients with mast cell disorders and recurrent anaphylaxis to Hymenoptera stings (42, 43).

It is of note that variations in metabolism of mediators, can influence not only the manifestations of anaphylaxis, but theoretically also the ability to recover from these manifestations in patients who have experienced anaphylaxis and survived the episod even though not treated (44).

Risk factors for severe anaphylaxis may be different also according to the trigger, as demonstrated in the case of HV allergy. Mastocytosis and monoclonal mast cell activation syndrome (MMAS) are well known risk factor for severe and even fatal anaphylaxis due to Hymenoptera stings, while this association is less clear for drug hypersensitivity (45).

Also risk factors for fatal anaphylaxis vary according to cause. For fatal drug anaphylaxis, previous cardiovascular morbidity and older age are risk factors, with beta-lactam antibiotics, general anesthetic agents, and radiocontrast injections the commonest triggers (46). For fatal food anaphylaxis, delayed adrenaline administration is a risk factor; common triggers are nuts, seafood, and in children, milk. For fatal venom anaphylaxis, risk factors include middle age, male sex, white race, cardiovascular disease, and possibly mastocytosis; insect triggers vary by region. Upright posture was reported a a feature of fatal anaphylaxis to both food and venom (46).

The so-called co-factors may explain why an allergen can either be tolerated or trigger a mild reaction or, in the same patients, induce a severe anaphylaxis. They may have two different effects: lowering the threshold, so that severe allergic reactions may be observed at much lower doses of allergen; increasing the severity, meaning that more severe reactions are elicited by the same dose of food or anaphylaxis is observed for the first time. Co-factors play a role in 30% of all anaphylactic reactions in adults and in 18% of children. The most frequent co-factors are exercise, non-steroidal anti-inflammatory drugs (NSAID), alchool, but menstruation, infections, medications other than NSAID (*e.g.* antiacids), extreme air temperature, cannabis use, stress and disruption of routine have been also reported (47).
The vast majority (90%) of excercise-induced anaphylaxis are related to food ingestion (FDEIA) (29, 48). Most of the data for FDEIA focusses on wheat triggers, being ω-5 gliadin the culprit proteins in most cases (49), even though in Mediterranea area lipid transfer proteins (LTPs) seem to be the most frequent sensitizer (50). In other studies (31) NSAID were the most frequent co-factor enhanced food allergy, followed by exercise, with LTP allergens being again the allergen most frequently involved.
The underlying mechanisms in FDEIA are still unclear and several hypotheses have been proposed, like exercise increasing gastrointestinal permeability, increasing activity of tissue transglutaminase in the gut mucosa, inducing blood flow redistribution, and finally, exercise increasing histamine release from basophils because of an increase of plasma osmolarity (51).
The frequent implication of cofactors in anaphylaxis highlights the importance of recognizing and including them into diagnostic workup (52).

Phenotypes and endotypes

Anaphylaxis involves the activation of multiple pathways (**table I**). Its endotypes can be divided according to the underlying mechanism and/or the effector cells involved in the reaction. A recent classification of anaphylaxis phenotypes has been proposed: type-I-like reactions, cytokine storm-like reactions, mixed reactions and complement-mediated reactions (1). Endotypes, underlying these phenotypes, are based on biological and molecular mediators supported by biomarkers. Type-I-like reactions are characterized by classical allergic symptoms (*e.g.* urticaria, pruritus, shortness of breath, throat tightness, nausea, vomiting, diarrhea, cardiovascular collapse), frequently (but not always) IgE-dependent, due to foods, drugs, Hymenoptera venoms, and environmental allergens. Cytokine storm-like reactions, as well as mixed reactions, are usually elicited by chemotherapy or biological agents and additionally induce some atypical symptoms such as chills, fever or pain. Finally, reactions mediated by complement can be induced by contrast dyes or dialysis membranes, among others, and provoke hypotension and desaturation.

Type-I-like reactions
These reactions represent the vast majority and include immune-mediated and non-immune-mediated pathogenesis. Antibody dependent anaphylaxis includes IgE-mediated and IgG-mediated reactions. In IgE-mediated reactions, anaphylaxis initiated by an allergen interacting with allergen-specific IgE (sIgE) bound to its high-affinity receptor (Fce RI) expressed on effector cells, mainly mast cell and basophils (44). Mast cell mediators responsable for allergic symptoms are represented by preformed mediators stored in the cytoplasmic granules released by degranulation such as histamine, the proteases tryptase and chymase, carboxypeptidase A and proteoglycans (with heparin as the major component); newly generated proinflammatory lipid mediators (*i.e.* prostaglandins, leukotrienes and PAF); and newly synthesized growth factors, cytokines and chemokines.
Mast cell, IgE and Fce RI depletion in animal models suppresses anaphylaxis (53-55), indicating that this pathway is crucial. In human anaphylaxis, the use of anti-IgE antibody, omalizumab, as an adjuvant treatment in food and venom immunotherapy reduces the risk (56, 57) and it prevents anaphylaxis in patients with systemic mastocytosis (58, 59). However, the presence of antigen-specific IgE antibodies does not indicate that the person necessarily will exhibit any, let alone severe, clinical reactivity to the recognized antigens. On the other hand, severe anaphylaxis can occur despite low levels or undetectable specific-IgE (sIgE) (44), suggesting the existence of IgE-independent mechanisms.
Not definitive evidence of IgG-mediated anaphylaxis in human subjects is present to date. It has been suggested that IgG-mediated anaphylaxis in humans requires considerably more antigen than IgE-mediated anaphylaxis, such as in reactions to infused drugs such as contrast media and antivenoms, due to the lower affinity of IgG binding by Fcg RIII than of IgE binding by Fce RI (60). Indeed, cases of anaphylaxis were reported after treatment with therapeutic monoclonal antibodies (mAbs) without detectable levels of anti-drug IgE (61, 62).
Basophils have been shown to be dispensable for IgE-mediated anaphylaxis but play a crucial role in IgG-mediated anaphylaxis in murine models, through their release of PAF and their ability to bind immune complexes via the low-affinity IgG receptor Fcg RIII (63). Recently, an emergency department study recruiting 31 patients with acute anaphylaxis, predominantly to Hymenoptera venom, showed that human anaphylaxis involves a substantial reduction in numbers of circulating basophils, which inversely correlate with serum CCL2 levels, a major basophil chemotactic factor, thus implying an important and specific role for basophils in the pathophysiology of human anaphylaxis (64).
Several drugs can induce direct nonimmunologic type-1-like activation of mast cells/basophils by basic secretagogues, including vancomycin, NSAIDS, opiates, fluoroquinolones and neuromuscular blocking agents. For example, opiates induce histamine release presumably through a mechanism that involves

opioid receptors in mast cell (65). Vancomycin is able to directly activate mast cell leading to histamine release in the 'red man syndrome' through a calcium-dependent mechanism that involves activation of phospholipase C and phospholipase A2 (66). An alternative mechanism, based on non IgE-mediated mast cells activation by means of the G-protein-coupled receptor X2 (MRGPRX2) has been identified. The receptor MRGPRX2, which is expressed on mast cells and other cells, has been shown to be activated by quinolone antibiotics, such as ciprofloxacin and levofloxacin; general anesthetics, such as atracuronium and rocuronium; icatibant; and other drugs with Tetrahydroisoquinoline (THIQ) motifs (67), even though its partecipation has not been confirmed in human subjects.

Contact and coagulation system can be activated in anaphylaxis through immunological and nonimmunological mechanisms (**table I**). The latter was related with oversulfated condroitin-contaminated heparin causing severe anaphylaxis through direct activation of factor XII (FXII) of the contact system and release of bradykinin (68). During the acute phase of human anaphylaxis, a strong consumption of contact system factors has been observed, associated with mast cell degranulation and increased plasma heparin levels, being heparin a potent FXII activator (69).

Finally, an activating mutation in c-KIT D816V promotes mast cell proliferation in patients with clonal mast cell disorders, including mastocytosis, in whom type-I-like anaphylaxis may occur with or without known triggers, with or without specific IgE sensitization (70).

Cytokine storm-like reactions

Cytokine storm-like reactions are caused by release of proinflammatory mediators, such as TNF-a, IL-1B, and IL-6, and the target cells include monocytes, macrophages, mast cells, and other immune cells with FcgR (11, 71). Triggers for these reactions include chimeric, humanized, and human mAbs and chemotherapy, including oxaliplatin. Reactions are characterized by chills, fever, generalized malaise followed by hypotension, desaturation, and cardiovascular collapse.

Similar to infusion-related reactions, cytokine-release reactions to mAbs can occur at first infusion, even though they have also been seen after several exposures. The difference between the two reactions is the self-limiting nature of infusion-related reactions on repeat exposure and the response to premedication (72-74). Premedication with anti-inflammatory COX-1 inhibitors and corticosteroids can decrease the intensity of cytokine release reactions but does not protect from severe reactions.

Mixed reactions

Mixed reactions with features of type I – and cytokine storm – like reactions can be seen with chemotherapy and mAbs in which pruritus, hives, and swelling are associated with chills, fever, hypotension, and desaturation (11, 72).

Complement-mediated reactions

Complement-mediated anaphylaxis may also occurr through immunological and nonimmunological mechanisms (**table I**). The anaphylatoxins C3a and C5a are potent inflammatory mediators generated upon activation of the complement cascade. Mast cell, ba-

Table I *- Immunologic and nonimmunologic pathways in anaphylaxis.*

Type of anaphylaxis pathway	Mechanism	Effector cell	Main mediator involved
Immunologic	IgE-dependent	Mast cell/basophil	Histamine, tryptase, chymase, carboxipeptidase,
	IgG-dependent	Basophil/Macrophage/Neutrophil	heparin, PAF PAF
	Complement system	Mast cell/macrophage	Histamine, PAF
	Contact system/Coagulation system (Kallikrein-FXII system)	Endotelial cells	Bradykinin
Nonimmunologic (*physical factors, ethanol, drugs*)	Complement system	Mast cells	
	Mast cell/basophil activation		
	- *Quinolones*	Mast cells	Histamine, tryptase
	- *Neuromuscolar blockers*	Mast cells	Chymase, Heparin, PAF
	- *NSAIDs*	Mast cells	
	- *Opiates*	Mast cells	
	- *Vancomicin*	Mast cells	
	- *Contact system/Coagulation system*	Endothelial cells	Bradykinin

sophils and monocytes/macrophages express receptors for C3a and C5a (75), and release histamine and/or PAF in response to exposure to these complement fragments. In human and mice anaphylaxis, complement activation by peanut (76, 77) or wasp-sting acted synergistically with IgE-dependent mast cell activation (78).
Activation of complement without immune complex formation has been shown to induce anaphylaxis in the absence of specific IgG or IgE. This mechanism has been described in association with hemodialysis, liposomal drug infusion, radiocontrast media, polyethylene-glycol infusion and micellar solvents containing amphiphilic lipids (*e.g.* Cremophor EL, diluent in propofol or paclitaxel) or liposomal doxorubicin (79-81).

Biomarkers

A biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (82). The ideal biomarker should be highly specific, sensitive, predictive, rapid and easy to measure, cheap, stable *in vivo* and *in vitro*, and noninvasive. Biomarkers in anaphylaxis hold the potential for improving diagnosis, stratification of severity, risk prediction, and therapeutic management, even tough they have no role for the moment in acute management.
Tryptase is considered a largely mast cell-derived product, being present in much lower amounts also in basophils. Mature β-tryptase is stored in mast cell granules and released on activation, such as in anaphylaxis, whereas α- and β-protryptases are secreted constitutively by mast cells, and therefore increased blood levels might indicate increased mast cell burden rather than anaphylaxis (83). Tryptase is much more stable than histamine and blood samples for its measurement are optimally obtained 15 minutes to 2-3 hours after symptom onset (84). Even though commercial methods measure total serum tryptase (immature and mature), this assay is still the best routine biomarker available to assess mast cell activation. Increased serum tryptase levels often support the clinical diagnosis of anaphylaxis from insect stings or injected medications and in patients who are hypotensive; however, levels are often within normal limits in patients with anaphylaxis triggered by food and in those who are normotensive (85). Furthermore, we must bear in mind that in anaphylactic reactions in which the main involved effector cell is not the mast cell, tryptase may not rise. Serial measurement of tryptase levels during an anaphylactic episode, and measurement of a baseline level after recovery are reported to be more useful than measurement at only one point in time (85). The "20% + 2 formula" has been validated in clinical practice and currently considered significant in clinical practice as a criterion of severe systemic mast cell activation and mast cell activation syndrome (MCAS) (86).
Histamine is also a marker of mast cell and basophil activation. Blood samples for measurement of its levels are optimally obtained 15-30 minutes after symptom onset. In fact, plasma histamine peaks within 5-10 min of the onset of symptoms and declines to baseline within 30 min as a result of rapid metabolism by N -methyltransferase and diamine oxidase (2).
Blood tests for other biomarkers, such as chymase (87), carboxypeptidase A3 (88), and CCL-2 (89) remain experimental.
Despite mast cell heparin has been reported to activate the plasma contact system during anaphylaxis, there are no available assays to measure it directly. Anti-Xa is an indirect measure of plasma heparin, but commercial assays are not sensitive enough (69).
PAF is a potent phospholipid-derived mediator implicated in platelet aggregation and it is secreted by mast cells, monocytes and fixed tissue macrophages (90). A limited number of reports have assessed concentrations of PAF or platelet-activating factor acetylhydrolase (PAF-AH), an enzyme responsible for the rapid degradation of PAF, after anaphylaxis in human subjects. In these reports circulating PAF levels were increased, and circulating PAF-AH activity was inversely correlated with the severity of anaphylaxis (39, 40, 91). One of the challenges with measurement of PAF and PAF AH in a routine clinical setting is its very short half-life and special sampling and transport precautions that are required thus making it an unattractive candidate for routine use (84).
CysLTs are potential mediators of anaphylaxis and are synthesized from arachidonic acid by a variety of cells, including mast cells, basophils, and macrophages (44). Several reports show that levels of some of these products, namely LTE4, 2,3-dinor-9a,11b-PGF2, and 9a,11b-PGF2, are increased during the onset of anaphylaxis (92, 93). However, like histamine, they have to be measured by 24-h urine collection, meaning that the sensitivity might be low.
Finally, levels of other serum inflammatory mediators, such as TNF-a, IL-6, and IL-1b, can be increased in patients with cytokine storm-like reactions and anaphylaxis, but their sensitivity or specificity has not been demonstrated. Among them, IL-6 has been pointed out as a potential biomarker for identifying and managing cytokine-release reactions (72).

Diagnosis

If the trigger is highly suspect, an accurate clinical history collection togheter with conventional diagnostic tools are sufficient to confirm the diagnosis. However, in many cases, especially in polysensitised patients, other procedure may help to confirm the cause, for istance component-resolved diagnosis (CRD), and basophil activation test (BAT).

Skin testing

The rationale of skin testing lies in the presence of an IgE-mediated pathogenesis and mast cell involvement. If performed within 2 to 4 weeks after anaphylaxis, skin tests are highly specific for type I reactions to Hymenoptera venoms (HV), foods, drugs (*e.g.*

beta-lactams, general anesthetics, platins). Unlike HV and drug allergies where both prick and intradermal test should be performed, only skin prick test (SPT) are indicated for food allergy diagnosis. Associations between SPT wheal size and severity of reaction on food challenge have been observed in a few studies, but these findings have not been consistent among studies (94).
In HV allergy, the sensitivity of the prick test is lower than the one of the intradermal test; intradermal tests should be performed even in case of positive prick test to identify correctly the cutaneous end-point which will be useful in VIT follow-up; in case of negative tests in subjects with a suggestive history, tests should be repeated after 1-2 months (95).
As for immediate hypersensitivity reactions to beta-lactams (BL), skin tests are more sensitive than in vitro test (96); even tought they become negative with time (93), they should be performed with great caution in case of anaphylaxis (97). It is important to enphasize that skin testing to penicillins requires major and minor determinants (93), the latter being available only in some European countries (98).
Skin testing in perioperative anaphylaxis are useful and all drugs/agents used before the reaction should be tested. An IgE-mediated mechanism has not been demonstrated for all drugs/agents, and a validation of skin testing is lacking. Testing and subsequent interpretation should be performed by experienced personnel using standardised concentrations as several drug groups, especially NMBAs and opioids, can cause irritant skin reactions (99).
For antibiotis other than beta-lactams as well as many other drugs, skin testing lacks well-defined predictive values. Positive skin tests with nonirritant concentrations are suggestive of drug-specific IgE; however, negative skin tests are less helpful due to unclear negative predictive values (100).
Finally, patients with cytokine storm-like reactions and complement activation are likely to have negative skin test results, indicating the lack of IgE participation, but patients with mixed reactions can have positive skin test results (71).

Serum specific IgE

Together with clinical history and SPTs, serum specific IgE (ssIgE) determination is commonly used for diagnosis of food allergy, thus reducing the need for food challenge (101). However, the clinical utility of sIgE for assessing risk of severe reactions has not been yet established (94).
The sensitivity of serological tests using HV whole extracts is generally lower than that of skin tests, and for *Vespula spp.* it is lower than the one for bee venom. In general, *in vitro* tests for the search of specific IgE toward the whole extract of venom can be negative in up to 20% of patients with positive skin tests, wher eas approximately 10% of patients with negative skin test are positive at *in vitro* test, suggesting to perform both tests (102).
SsIgE determination in BL allergic reactions has to be performed togheter to skin testing since cases with immediate hypersensitivity reactions to BL with negative ST and positive ssIgE have been reported (103). As for ST, also the sensitivity of ssIgE decreases with time, thus suggesting to be performed as soon as possible after the reaction (104). In patient with BL anaphylaxis, clearly positive ssIgE can be useful for avoiding both ST and drug provocation test (DPT) (105).
SsIgE quantification can be used for a limited number of drugs in the perioperative setting. The reported sensitivity and specificity are very good for sIgE for latex and chlorhexidine, but show great variation for NMBAs and morfines (99).
In general, ssIgE determination cannot be used for the evaluation of the majority of drugs able to induce IgE-mediated reactions (104).

Component Resolved Diagnosis (CRD)

CRD, using single molecules or panels of allergens, is a new tool which has revolutionized allergy diagnosis in recent years, helping to improve diagnostic accuracy, and in some cases providing information on risk assessment and consequently on management (106). Nevertheless, we must note that is only able to assess sensitization and not clinical reactivity.
As for food allergy, CRD may be helpful in complicated, polysensitized patients, mixed food intake, as well as in cofactor-enhanced food allergy (ω-5-gliadin, and nonspecific lipid transfer proteins (nsLTP) (31, 50, 107) and in red meat delayed anaphylaxis (α-Gal) (108).
Anaphylaxis has been associated with certain components, such as seed storage proteins (2S albumins, 7S vicilins, and 11S legumines) or nonspecific lipid transfer proteins (nsLTPs) (109), even tough severity risk attributed to specific molecules may vary according to other factors such as geographic variations, degree of allergen exposure, cosensitizations, and cofactors (110).
In the field of HV allergy, CRD may discriminate between primary sensitization and cross-reactivity in patients with double/multiple positivity in diagnostic tests with whole extracts (especially in case of bee and yellow jacket double sensitization), allowing the specialist to choose the most suitable venom for specific immunotherapy (VIT), avoiding unnecessary VIT and reducing the risk of side effects (111). CRD may be useful in patients with negative allergy tests and a proven history of a previous systemic reaction, including those with mast cell disorders, who could benefit from VIT. In honeybee venom allergy, different sensitization profiles have been identified, which could be associated with a greater risk of VIT failure or treatment side effects (112, 113).
Latex allergy may be an important cause of anaphylaxis, even though its incidence has decreased in the last 10 years. In this regard, monosensitization to Hev b 8 (profilin) suggests cross-re-

active and asymptomatic sensitization, whereas markers of genuine allergy like Hev b 1, Hev b 3, Hev b 5, and Hev b 6, may potentially induce severe reactions, thus making it necessary to apply avoidance measures (114).
A diagnosis of idiophatic anaphylaxis (IA) is based on exclusion of known triggers of anaphylaxis, as well as conditions that can masquerade as anaphylaxis (115). The diagnostic utility of an allergen microarray (ImmunoCAP ISAC) in the detection of possible allergenic triggers in patients with unexplained anaphylaxis has been evaluated, showing evidence of sensitization to newly identified allergens (mainly wheat, shrimp and peanut) that had not been detected during routine allergy workup, even tough an allergen challenge procedure to confirm the diagnosis was not performed (116). Finally, CRD has no application in the field of drug allergy.

Basophil Activation Test (BAT)

Among blood-cell based tests, BAT is the most widely used in Europe for diagnostic purposes, in selected situations, and in highly specialized laboratories.
The BAT has shown to be more accurate than IgE sensitization tests and able to distinguish individuals that were clinically allergic from those who were tolerant to some food like peanut, showing a high specificity, thus dispensing from doing food provocation test (117).
The BAT can identify approximately two thirds of HV allergic patients with positive history and negative skin and serological tests (118). It is also recommended in patients with double positive results and inconclusive results of *in vivo* or *in vitro* tests with recombinant allergens (119). The role of BAT as a diagnostic tool in patients with mastcell disorders and negative venom-specific IgE and skin test results is still controversial (95).
Taking into account the diagnostic difficulties specific to drug allergy and risks related to provocation test in the case of anaphylaxis, the BAT should theoretically represent a safer opportunity to be improved. Several studies over the last 15 years have reported the diagnostic accuracy of BAT for allergy to a range of drugs including betalactams, quinolones, platins, and neuromuscular blocking agents (NMBAs) (120).

Provocation test

The food allergen provocation test (FAPT) provides a gold standard diagnostic for food-related adverse reactions leading to appropriate food avoidance (101). However, a severe anaphylactic reaction to a given food with highly positive IgE tests, and a history of several reactions to the same food, will in most cases not need a FAPT (121). The use of CRD in some cases may help to grading the risk of a positive reaction to FAPT (122, 123).
The sting challenge has a low predictive value as a patient with a negative test might still react on a field sting. It differs from other provocation tests in that incremental allergen exposure is not possible and insect biology and several other factors may influence the test result. Even if it may have an indication during VIT to verify its efficacy, it is contraindicated in untreated patients and after stopping the treatment (124).
Drug provocation test (DPT) is a part of drug allergy workup. The majority of the studies were perfomed with BL. The European guidelines and the U.S. practice parameter give different indications to the DPT with BL, and a significant heterogeneity in European current practice has been recently demonstrated (105). However, in the case of anaphylaxis DPT should be avoided regardless of the type of drug involved.

Idiopathic anaphylaxis and mastcell diseases

Idiopathic anaphylaxis is a diagnosis of exclusion and mandates careful consideration of all recognizable and rare causes of anaphylaxis (115, 125, 126). The clinical manifestations and management of acute episodes are the same as for other forms of anaphylaxis. A good clinical history is paramount to direct further investigations. Idiopathic anaphylaxis represents an opportunity for identification of previously unrecognized novel triggers and also for identification of mastocytosis or clonal mast cell disorders. Particular attention should be paid to "hidden allergens," cofactors (*i.e.* wheat-dependent exercise-induced anaphylaxis), galactose alpha-1,3 galactose (a carbohydrate contained in red meat) allergy, pigeon tick bite (Argax reflexus), and Anisakis simplex allergy (115). Out of the 30 cases of IA with no signs of cutaneous mastocytosis, 47% were found to have an aberrant MC population and were subsequently diagnosed with clonal mastcell (MC) disorder (127). Similarly, the presence of a clonal mast cell population with a diagnosis of IA was reported, in whom there were no features of urticaria pigmentosa or histological evidence for systemic mastocytosis on bone marrow (BM) biopsy (128). These findings demonstrate a need for robust criteria for BM examination in cases of suspected clonal MC disorders in the context of IA. The only available validated tool is the Spanish Network on Mastocytosis (Red Española de Mastocitosis - REMA) scoring system, based on a combined clinical (*i.e.* gender and clinical symptoms) and laboratory (baseline tryptase value with "cut-off" of 15-25 ng/mL) parameters, to predict underlying MC clonality in patients presenting with systemic MC activation symptoms, including anaphylaxis (129).
Other differential diagnoses include "allergy-mimics" such as asthma masquerading as anaphylaxis, undifferentiated somatoform disorder, panic attacks, globus hystericus, vocal cord dysfunction, scombroid poisoning, vasoactive amine intolerance, carcinoid syndrome and phaeochromocytoma (115). Diagnosis must be revisited in cases with recurrent episodes, where there is paucity of clinical signs and/or in the context paucity of refractoriness to corticosteroid therapy.

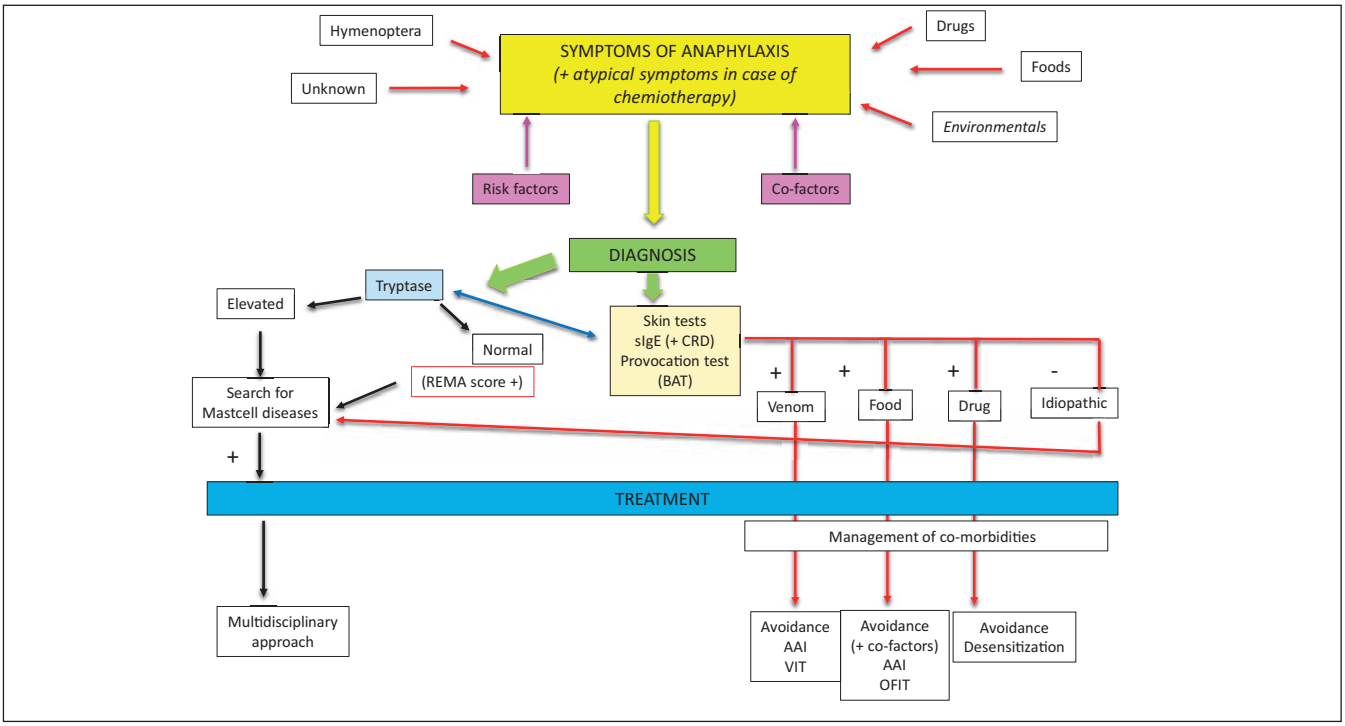
Treatment and management

Patients with a history of anaphylaxis have an increased risk of severe reactions in the future, thus indicating that secondary prevention measures are of paramount important, as suggested by different international guidelines (1, 2). The most important preventive measures include the identification, and consequent avoidance of triggers and co-factors, the recognition by the patient of the first symptoms indicative of anaphylaxis, the availability of an (AAI) and management training, optimal management of relevant co-morbidities, venom specific immunotherapy, food oral immunotherapy, and drug desensitization, when indicated (**figure 1**).

Acute treatment: adrenaline

The acute management and treatment of anaphylaxis depend on early recognition and prompt use of adrenaline, as it is the first-line treatment of anaphylaxis and it is life-saving (1-6). Treatment of anaphylactic reactions in the hospital setting should adhere as closely as possible to guidelines. Indeed, only 27.1% of European patients with anaphylaxis treated by a health professional received adrenaline and, in total, 10.5% received a second dose (130). Interestingly, successful administration was less frequent in German-speaking countries (minimum 19.6%) than in Greece, France, and Spain (maximum 66.7%). Nevertheless, over the last decade adrenaline administration from a health professional almost doubled to reach 30.6% in 2015-2017, probably reflecting improved guideline distribution and awareness (130).
All patients with a history of anaphylactic reaction should be provided with adrenaline autoinjectors to be injected into the vastus lateralis muscle (1-6). There are six absolute indications for a prescription of an adrenaline auto-injector (1): previous anaphylaxis with food, latex, aeroallergens such as animals or other unavoidable triggers; previous exercise-induced anaphylaxis; previous IA; co-existent unstable or moderate to severe, persistent asthma with food allergy; venom allergy in adults with previous systemic reactions (unless receiving maintenance VIT) and children with more than systemic cutaneous reactions; and underlying mast cell disorder and any previous systemic reaction. European Guidelines (1) suggest also to consider prescribing at least one adrenaline auto-injector with any of the following additional factors (especially if more than one is present): previous mild-to-moderate allergic reaction to peanut and/or tree nut; teenager or young adult with a food allergy; emote from medical help and previous mild-to-moderate allergic reaction to a food, venom, latex, or aeroallergens; previous mild-to-moderate allergic reaction to traces of food. Indications for prescription of a second adrenaline auto-injector have been also suggested (1). Of note, the European Medicines Agency (EMA),

Figure 1 *- Algorithm for the management of anaphylaxis in clinical practice.*



VIT (venom immunotherapy); AAI (adrenaline autoinjector); OFIT (oral food immunotherapy); BAT (basophil activation test); CRD (component resolved diagnosis).

after evaluation of all available data, recommended healthcare professionals to prescribe two autoinjectors (European Medicine Agency, 26 June 2015. EMA/411622/2015).
According to the data from the Anaphylaxis Registry (131), an AAI prescription was offered to only 37% of the patients ouside specialized centers compared to 84% of the patients in specialized allergy centers, highlighting the need of better education for primary healthcare and emergency physicians to follow guidelines. In the multivariate analysis, the elicitor of the reaction (less prescriptions in patients with food allergy than in those with venom allergy), age of the patient (less prescriptions in babies and elderly patients), mastocytosis as comorbidity, severity of the reaction, and reimbursement/ availability of the autoinjector influence physician's decision to prescribe one (131).
An integral part of the anaphylaxis action plan is represented by advising patients to carry the device with them at all times, as well as instructing the patient on how to use the autoinjector through educational material and practical training (1, 2). Nevertheless, in Europe few lay- or self-treated cases receive an autoinjector (14.7%), even tough clinical severity considerably influence the likelihood of receiving adrenaline (130). Moreover, it is alarming that no change in successful administration by lay emergency respondents was found over the last 10 years (130), underlining the persistence of several gaps in the management of severe allergic reactions (132).
Although many patients are afraid to use their AAI (133), no significant adverse effects have been reported, with the exception of the known onset of tachycardia, tremors, and peripheral vasoconstriction (134).
Finally, corticosteroids and antihistamines are not lifesaving, they have not been demonstrated to prevent biphasic anaphylaxis and their use should never delay adrenaline administration (1, 2).

Specific immunotherapy and desensitization

Specific subcutaneous immunotherapy for hymenoptera venom is the only treatment able to protect patients from systemic reactions after subsequent stings (protection against reported in 91-96% of cases, 77-84% for bee allergy) (135). Nonetheless, VIT offerslong lasting protection upon re-sting even after discontinuation of treatment, and increases dramatically the quality of life of HVA patients (95, 135). It is effective and safe even in patients with mastcell diseases (136). As rush and ultra-rush protocols offer rapid protection from re-sting as early as the maintenance dose is achieved, they should be offered to patients with severe reactions.
Even though oral food immunotherapy may increase the amount of a tolerated dose over time (137), and enhances sustained unresponsiveness that persists after cessation of therapy (138), there are currently no established oral immunotherapy treatment protocols for food-induced anaphylaxis. Since significant systemic side-effects can occur, currently this treatment is not recommended in clinical practice (101).

Drug desensitization to drug is a highly effective readministration strategy for those patients who develop hypersensitivity reactions to their needed medications, like chemotherapeutic agents, mAbs (72, 139, 140), antibiotics (141) and many other drugs (142). Of note, it has been documented that carboplatin-desensitized patients had a non-statistically significant lifespan advantage over nonallergic controls, indicating that the efficacy of carboplatin was not reduced in allergic patients and that desensitization protocols are as effective as regular infusions (140). Even patients presenting with type I and cytokine-release reactions to mAbs are thought to be candidates for desensitization (11).
The anti-IgE mAb Omalizumab has been shown to be a successful treatment for reducing the number and severity of anaphylactic reactions in association with VIT (58) or with food oral immunotherapy (143).

Conclusions

Anaphylaxis is the most severe allergic reaction, it involves multiple organ systems, can be caused by a number of triggers and conditions, and be deadly. Although rapid advances in allergy and immunology concerning the identification of new allergens, biomarkers and cofactors, as well as the availability of new diagnostic tools, there are still many gaps in evidence and knowledge. There is still much to be done to identify genetic and epigenetic markers and cofactors for determining risk of anaphylaxis to specific allergens, performing an individual risk assessment, and preventing future episodes by developing personalized risk reduction strategies. Gaps in knowledge and anaphylaxis management have been observed at different levels, at the level of patients, community as well as physicians (131). Diagnosis of anaphylaxis, evaluation of the severity of the allergic reaction, and the use of adrenaline is insufficient for many physicians and a gap between best practice and Emergency Department (ED) care has been also reported. These findings highlight the need for an easier definition of anaphylaxis especially for non-allergists to improve the diagnosis and consequently the appropriate treatment with adrenaline.
Further identified gaps in the management of anaphylaxis include infrequent or delayed use of AAI by the patients for acute allergic reactions, as well as inadequate AAI training, and prescription rates for patients at risk (144). A recent review of a number of English language anaphylaxis management plans underlines a wide variety of content, with no plans having 100% of the recommended material (145). Therefore, more appropriate training for patients, families and caregivers of patients are necessary.
Finally, very few studies are being designed to determine how to increase adherence to existing anaphylaxis guidelines and best practice through integrated knowledge translation strategies.

Conflict of interests

The authors declare that they have no conflict of interests.

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Can concurrent lower gastrointestinal manifestations help the timely diagnosis of small intestinal bacterial overgrowth in CVID patients?

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KEY WORDS

Common Variable Immunodeficiency (CVID); Small Intestinal Bacterial Overgrowth (SIBO); chronic diarrhea; hydrogen breath test; primary immunodeficiency; gastrointestinal symptoms.

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Summary

Introduction and objective. *Gastrointestinal complications are considered as one of the most common manifestations in patients with Common Variable Immunodeficiency (CVID). These complications can result from Small Intestinal Bacterial Overgrowth (SIBO). Hydrogen breath test is extensively used to diagnose SIBO. The objective of this study was to evaluate the prevalence of SIBO using the Hydrogen Breath Test (HBT) in patients with CVID.* ***Materials and methods.*** *Twenty-seven patients with CVID entered this cross-sectional study. Demographic and lower gastrointestinal symptoms were recorded in a check list. Hemoglobin level was measured in all patients. The concentration of IgA and IgG was assessed using nephelometry. Moreover, SIBO was detected by means of Glucose hydrogen breath test.* ***Results.*** *The mean (± SD) age of the patients was 35.25 (± 11.69) years. Twenty patients (74.1%) manifested at least one lower gastrointestinal symptom. The most frequent lower gastrointestinal manifestations were bloating (66.7%) and chronic diarrhea (40.7%), respectively. IgA level less than 10 mg/dl and IgG level less than 600 mg/dl were determined in 77.8% and 25.9% of patients, respectively. Positive HBT was detected in 40.7% (n = 11) of the patients. In the positive HBT group, bloating, chronic diarrhea and abdominal pain were the most common lower GI manifestations. There was no significant difference in terms of age, BMI, IgA level, and duration of CVID between the positive and negative HBT groups. The significant association of co-occurrence of anemia and abdominal pain with positive HBT (positive predictive value: 100%) might be considered as a clue to SIBO diagnosis.* ***Conclusions.*** *Regarding the high prevalence and non-specific manifestation of SIBO, it is suggested to consider concurrent symptoms in patients with CVID to manage the timely and precise diagnosis of SIBO.*

Introduction

Primary Immunodeficiency Diseases (PIDs) as a group of heterogeneous disorders are characterized by inability of the immune system to protect the body against foreign hazards (1, 2). Gastrointestinal (GI) problems are ranked as the second common manifestations in PID patients (2). It is noteworthy that GI complications are variable in different PIDs as well as different individuals (2).

Common Variable Immunodeficiency (CVID) as a heterogeneous Primary Immunodeficiency Disease (PID) with a prevalence of 1 in 25,000 to 50,000 is characterized by hypogammaglobulinemia, failure to produce immunoglobulin against vaccines and recurrent bacterial infections (3-5). Increased susceptibility to bacterial infections in respiratory and gastrointestinal tracts has been reported in more than 90% of patients with CVID (6). Given that the gastrointestinal tract contains a lymphatic system, it can be involved up to 50% of primary immunodeficiency diseases (5). Gastrointestinal manifes-

tations are infections, inflammation, autoimmunity, and cancers (5). These gastrointestinal symptoms have different pathogenesis and do not often improve following conventional treatments (5). Previous studies have reported acute or chronic diarrhea as the most frequent gastrointestinal manifestations ranged from 20 to 60% in patients with CVID (7-9). The causes of chronic diarrhea in these patients are microbial and non-microbial. One of the major causes of diarrhea in these patients is small intestinal bacterial overgrowth that can be managed by broad-spectrum antibiotics (10, 11).

Although Small Intestinal Bacterial Overgrowth (SIBO) is common in patients with CVID, its diagnosis is difficult owing to continuous or interrupted use of antibiotics (11). The small intestine aspirate and its culture as well as Hydrogen Breath Test (HBT) are applied as two current clinical tests to detect SIBO. Indeed, HBT is a cheap, easy to use and non-invasive test. Moreover it is regarded as the most popular test to diagnose SIBO in high-risk patients (12). The aim of this study was to assess the prevalence of SIBO as one of the reasons for chronic diarrhea in patients with CVID.

Materials and methods

This cross-sectional study was conducted in Rasool Akram Hospital from 2009 to 2019. All patients with CVID enrolled in the study, according to the diagnostic criteria of ESID 2014 (13). This study was approved by the Ethics Committee of Iran University of Medical Sciences (N: IUMS.FMD.REC.1397.177). The inclusion criterion required was the correct preparation prior to perform a hydrogen breath test.

All characteristics including demographic data and lower gastrointestinal symptoms (chronic diarrhea, bloating, abdominal pain, constipation and steatorrhea) were recorded for all patients. The concentration of hemoglobin was assessed. Furthermore, the level of IgA and IgG was measured using nephelometry (Eppendorf, Ecom-EG125 system, Germany) during Ig replacement therapy and before receiving the last dose of IVIG. To assess SIBO, HBT was performed using Fischer system (Lacto FAN2, Germany).

Hydrogen Breath Test (HBT)

To do this test, patients were asked to consider some precautions including (1) not taking antibiotics for 4 weeks prior to the test, (2) avoidance of carbohydrates foods (such as bread, potato and spaghetti) for 24 h before the test, (3) not taking any fiber supplements and laxatives 24 h before the test, (4) fasting for 8-12 h before the test, (5) avoidance of smoking, sleeping and physical activity 30 minutes before and during the test (6) and the use of chlorhexidine mouthwash before the test.

The baseline of breath hydrogen was evaluated for all patients. Then, 75 g of glucose dissolved in the water was ingested by patients. The HBT was repeated every 20 minutes for 120 minutes. An increase of 20 part per million (ppm) in hydrogen compared to baseline at least two times during 90 minutes was considered to be a positive test (12).

Statistical analysis

To analyze the data, a set of quantitative and qualitative statistical tests available in the SPSS software version 20 (IBM, Armonk, NY, USA) was used. To describe the categorical variables, the frequency and percent were determined. Mean ± SD and median (25^{th} percentile (Q1), 75^{th} percentile (Q3)) were calculated to describe the normal and non-normal quantitative variables, respectively. In order to compare the mean difference of a quantitative variable between two dependent groups, Independent t-test or Mann-Whitney test were applied. To evaluate the relationship between two categorical variables, Chi Square or Fisher Exact test were used. The significant level was considered less than 0.05. Graphpad Prism 5 (Graphpad Software Inc., La Jolla, CA, USA) software was applied for drawing the graphs.

Results

The mean (± SD) age of the 27 included patients was 35.25 (± 11.69) years. Sixteen subjects (59.3%) were female. The mean (± SD) of Body Mass Index (BMI) was 22.27 ± 3.89. The median (Q1, Q3) duration of CVID was 9 (4, 14) year. Seven patients (25.9%) had a BMI less than 20. Anemia was detected in 33.3% (n = 9) of the patients. Among 9 patients with anemia, four and five patients had microcytic anemia and normocytic anemia, respectively. In addition, 77.8% and 25.9% of the patients with CVID showed IgA level less than 10 mg/dl and IgG level less than 600 mg/dl, respectively.

Twenty patients (74.1%) presented at least one lower gastrointestinal symptom. The frequency of lower gastrointestinal manifestations is shown in **figure 1**. As the figure demonstrates, the most frequent lower gastrointestinal symptoms were bloating (66.7%) and chronic diarrhea (40.7%), respectively.

Table I shows findings of GI symptoms, clues for malabsorption (anemia, BMI), immunoglobulin deficiency (IgG, IgA) and GI comorbidities in the positive and negative HBT group. Hydrogen Breath Test (HBT) was positive in 40.7% (n = 11) of the patients. In the CVID patients with positive HBT, bloating, chronic diarrhea and abdominal pain were the most common lower GI manifestations. No significant difference was found in terms of age, BMI, IgA, IgG and duration of CVID according to the result of HBT. The gastrointestinal comorbidities such as Inflammatory Bowel Diseases (IBD) and polyposis of the ileum were reported through records of patients. Among the studied patients, two IBD cases with positive HBT, two cases with polyposis of the ileum (one positive HBT) and two cases with Nodular Lymphoid Hyperplasia (NLH) (one positive HBT) were reported.

Some SIBO-associated symptoms such as abdominal pain and bloating were higher in HBT-positive group compared to HBT-negative group. A significant relationship was found between co-occurrence

Figure 1 *- The frequency and percent of lower gastrointestinal manifestations in patients with CVID.*

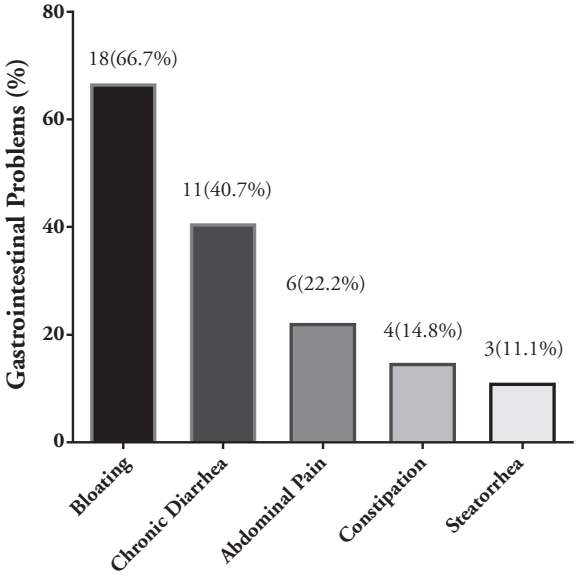

Table I *- The characteristics of CVID patients with positive and negative HBT results.*

		Total	Positive HBT	Negative HBT	P value
Gastrointestinal Symptoms N (%)	Bloating	18 (66.7)	8 (72.7)	10 (62.5)	0.69
	Chronic diarrhea	11 (40.7)	6 (54.5)	5 (31.3)	0.26
	Abdominal pain	6 (22.2)	4 (36.4)	2 (12.5)	0.18
	Constipation	4 (14.8)	1 (9.1)	3 (18.8)	0.62
	Steatorrhea	3 (11.1)	2 (18.2)	1 (6.3)	0.54
Clues for malabsorption N (%)	Anemia	9 (33.3)	5 (45.5)	4 (25)	0.41
	BMI < 20	7 (25.9)	3 (27.3)	4 (25)	1.00
Immunoglobulin deficiency Median (Q1, Q3)	IgG (mg/dl) Median	714 (500, 770)	760 (460, 830)	700 (525, 736)	0.29
N (%)	IgG < 600 mg/dl	7 (25.9)	3 (27.3)	4 (25)	1.00
	IgA(mg/dl)	2 (0,6)	3 (0,6)	1 (0,15.5)	0.86
	IgA < 10 mg/dl	21 (77.8)	9 (81.8)	12 (75)	1.00
GI comorbidities N (%)	Inflammatory Bowel Diseases (IBD)	2 (7.4)	2 (18.2)	0 (0)	0.15
	Polyposis of the ileum	2 (7.4)	1 (9.1)	1 (6.3)	1.00
	Nodular lymphoid hyperplasia	2 (7.4)	1 (9.1)	1 (6.3)	1.00

CVID (Common Variable Immunodeficiency); HBT (Hydrogen Breath Test); GI (Gastrointestinal); 25th percentile (Q1); 75th percentile (Q3).

of anemia and abdominal pain with positive HBT (P = 0.05) (**table II**). A positive predictive value of 100% was achieved for anemia accompanied with abdominal pain. Taking into account the small number of studied cases and the statistical results, which do not reach significance, it would be better to more cautiously state that HBT-positive patients tend to be those patients with IgG less than 600 mg/dl and abdominal pain. Other combinations (abdominal pain + bloating, anemia + bloating, chronic diarrhea and IgA < 10 mg/dl, abdominal pain and IgA < 10 mg/dl) seem to be less specific, although greater numbers are associated with HBT positivity.

Discussion

Herein, we assessed the prevalence of Small Intestinal Bacterial Overgrowth (SIBO) by using the Hydrogen Breath Test (HBT) in patients with CVID. As the results show, bloating and chronic diarrhea were found as the most common lower gastrointestinal symptoms in patients with CVID. Moreover, SIBO was detected in more than 40% of the patients.

As the literature review shows, the frequency of SIBO has been underestimated in the last decades, resulting in misdiagnosis and remarkable clinical complications in patients (12, 14). There were some reasons for explaining this issue, including a wide spectrum of SIBO-related non-specific clinical symptoms, lack of consensus guidelines for diagnosis as well as a gold standard test to precisely to detect the frequency of SIBO. These issues could make the diagnosis and treatment more complicated and challenging. In other words, the accurate prevalence of SIBO and its association with a specific disease is unclear (12) . Although SIBO is one of the main and common causes of chronic diarrhea in patients with CIVD, it is difficult to be diagnosed because of constant or irregular antibiotic usage in these patients (11).

In the current study conducted on patients with CVID, no significant relationship was found between the frequency of SIBO and age, gender, and duration of CVID. According to the literature, a relationship was found between the prevalence of SIBO and age and gender (15).

In this study, lower gastrointestinal manifestations were observed in 74.1% of the patients and bloating and chronic diarrhea were the two most frequently reported lower GI symptoms. In line with our findings, the small intestinal bacterial overgrowth often leads to bloating and chronic diarrhea (14). Moreover, in HBT-positive group, 81% of patients manifested symptoms associated with SIBO, while the others (19%) manifested none. Given that the symptoms related to SIBO vary in severity from asymptomatic to severe, it can be presumed that in asymptomatic cases, strains grown do not affect the gastrointestinal mucosa (12).

Chronic diarrhea was seen in 40.7% of our patients. Hermanszewsk *et al.* reported a similar frequency (40%) of chronic diarrhea in patients with CVID (16). On the other hand, a higher prevalence of chronic diarrhea (up to 60%) was found in studies by Hemans *et al.* (17) and Atarod *et al.* (18). In HBT-positive cases, bloating was reported as the most common GI manifestation. In line with this study, Mattsson *et al.* performed breath test in a population of adult patients, who indicated bloating as the most common symptom. As their study demonstrated, 46% of patients showed positive breath test (19).

In severe cases, patients with SIBO may develop severe weight loss as a result of diarrhea, insufficient food intake, and most notably nutrient malabsorption. Deficiency of vitamins, such as B12 results in anemia in these patients (10, 20). As the present study revealed, microcytic or normocytic anemia was detected in 33.3% and 45.5% of CVID patients accompanying with positive HBT. Although Vitamin B12 deficiency may result in a megaloblastic anemia in severe cases, iron deficiency has also been described in SIBO but the mechanisms need further elucidation (12). On the other hand, our cases have a chronic disease, and they may have anemia because of it.

Table II *- The relationship between co-occurrence of clinical symptoms and Small Intestinal Bacterial Overgrowth (SIBO) in patients with CVID results.*

	Total N (%) (95% CI)	**Positive HBT N (%)**	**Negative HBT N (%)**	**P value**
Abdominal Pain and Anemia	3 (11.1) (5.06, 17.14)	3 (100)	0 (0)	0.05
Abdominal Pain and Bloating	6 (22.2) (6.53, 37.87)	4 (66.7)	2 (33.3)	0.18
Anemia and Bloating	6 (22.2) (6.53, 37.87)	4 (66.7)	2 (33.3)	0.18
Chronic Diarrhea and IgA < 10	9 (33.3) (15.6, 51)	5 (55.6)	4 (44.4)	0.41
Chronic Diarrhea and IgG < 600	2 (7.4) (-2.47, 17.27)	1 (50)	1 (50)	1.00
Abdominal pain and IgA < 10	4 (14.8) (1.5, 28.1)	3 (75)	1 (25)	0.27
Abdominal pain and IgG < 600	2 (7.4) (-2.47, 17.27)	2 (100)	0 (0)	0.15

CVID (Common Variable Immunodeficiency); HBT (Hydrogen Breath Test).

There is a positive relationship between anemia and other symptoms associated with SIBO since the co-occurrence of anemia with abdominal pain was significantly higher in patients with SIBO. Positive predictive value of 100% was obtained for anemia accompanied by abdominal pain, which can provide a clue to SIBO diagnosis. A higher incidence of SIBO in patients with abdominal pain + bloating, anemia + bloating, abdominal pain + IgG < 600, IgA less than 10 mg/dl + diarrhea as well as IgA less than 10 mg/dl and abdominal pain was observed. This finding is consistent with previous studies suggesting that GI infections are more common in patients with undetectable serum IgA level (21).

In this study, two limitations need to be considered. Firstly, the frequent use of antibiotics in patients with CVID owing to underlying diseases decreased the number of the patients able to perform the HBT, thereby leading to small sample size. Secondly, the exclusion of some patients due to respiratory problems and inability to perform HBT, reduced the number of the patients. Moreover, cross-sectional design of the study might also underestimate the number of patients with SIBO.

Conclusions

Regarding the findings of the current cross-sectional study, bloating and chronic diarrhea were considered as the most common lower gastrointestinal symptoms in patients with CVID and SIBO was detected in more than 40% of these patients. Moreover, a 100% positive predictive value for anemia accompanied with by abdominal pain. Notwithstanding the limitations due to the small number of cases, gastrointestinal symptoms and routine clinical evaluation arise as potential valuable diagnostic clues. Timely and precise diagnosis of SIBO may lead to appropriate management of manifestations using broad-spectrum antibiotics and thereby preventing weight loss and malabsorption stemmed from diarrhea and finally improve the quality of life in these patients.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Allergic sensitization profiles among the foreign-born population in the north of Madrid

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Summary
Background. *We assessed differences in allergic sensitization and clinical characteristics in a foreign-born population.* **Methods**. *Prospective, observational, descriptive study of patients aged > 12 years who were seen at the Department of Allergy, La Paz Hospital (Madrid, Spain), between January 2017 and December 2018. Patients were classified by geographical origin and ethnicity.* **Results**. *We included 150 patients (110 female) with a mean age of 38.38 years. Mean time to onset of respiratory symptoms after immigration was 8.47 years. Significant differences were observed between ethnic groups (p = 0.007). The most frequent sensitization was to grass pollen (75.2%), which was more common in South American patients (p = 0.005). We found that 59% of patients were sensitized to Cupressus and Olea pollen (higher in Asian patients, p = 0.032 and p = 0.049).* **Conclusions**. *Allergic sensitization in the foreign-born population was similar to that of the autochthonous population although differences between the groups were identified.*

Introduction

The increased prevalence of respirator allergy is associated with the influence of genetic and environmental conditions (1). In developing countries, this increase has recently been associated with industrialization and lifestyle changes in the last decades (2). In Asia and Africa, this change has occurred alongside marked expansion of urban areas and rural exodus (3).
Rhinitis, whether associated with asthma or not, is one of the most common allergic diseases in our setting. Its prevalence worldwide is estimated to be between 10% and 30% and in Spain, around 22%. The prevalence of asthma is 4%-10% worldwide and approximately 7% in Spain (4). According to published data, 20%-50% of patients with rhinitis in Spain also have asthma, and 30%-90% of patients with asthma have rhinitis. (5).
The statistics show that foreign-born inhabitants accounts for 5% of the world's population (6). Both adults and children, are exposed to allergens and lifestyles that differ from those of their countries of origin, thus highlighting the implication of the environment on the development of allergic diseases (7). Children born shortly after settling in their new country have a similar allergic profile to that of the autochthonous population (8). Few published studies analyze the profile of sensitization to relevant respiratory allergens in specific populations, and data are contradictory. A study in Italy revealed that immigrants become sensitized to multiple allergens more frequently than the autochthonous population and are especially sensitized to local allergens (8), while a study in Spain shows that the pattern of sensitization differs between the native and the migrant populations (9). We explored potential differences between allergic sensitization profiles in the foreign-born population living in an area of Madrid, Spain, as well as the clinical characteristics of allergic respiratory disease (rhinitis, conjunctivitis and/or asthma) according to the patient's area of origin.

Methods

We performed a prospective, observational, descriptive study to explore allergic sensitization patterns and the clinical characteristics of allergic respiratory diseases in foreign-born patients aged > 12 years who attended the outpatient Allergy clinic. They were consecutively included between January 2017 and December 2018. Patients were classified by ethnic group (Caucasian, Arab, Black, Asian and Latino) and their area of origin (Europe, Asia, Africa, and South America). The study was approved by the local Clinical Research Ethics Committee (HULP PI-2528). All patients signed an informed consent document. Respiratory allergy was diagnosed using a standard work-up for allergic diseases (rhinitis with or without asthma), according to the updated ARIA guidelines for rhinitis (10) and the GINA guidelines for asthma (11). We included demographic factors, comorbidities, allergic background and clinical reports. A detailed physical examination was also performed. The quality of life of patients with rhinoconjunctivitis was assessed based on ESPRINT-15 (scores from between 0 (no limitation) to 6 (maximum limitation)) (12). Control of asthma was assessed using the self-reported Asthma Control Test (ACT) score ranging from 5 (poor control of asthma) to 25 (complete control of asthma) (13). Skin prick tests were performed with a panel of common aeroallergens. Histamine (10 mg/mL) was used as a positive control, and 0.9% saline solution was used as a negative control. A positive result was defined as a wheal > 3 mm larger than that of the negative control. Serum specific IgE was also assessed (ImmunoCAP, ThermoFisher Scientific, Sweden), with a value > 0.35 kU/L being considered a positive result. Lung function (including spirometry and the bronchodilator test), fractional exhaled nitric oxide, and eosinophil count in peripheral blood were assessed according to usual clinical practice. The statistical analysis was performed using SPSS Statistics 26.0 for Windows. Continuous variables were described as mean, median, interquartile range, standard deviation, and range. Discrete variables were presented as a frequency distribution, and percentages, and 95% confidence intervals were calculated where necessary. Comparisons were made using 2-tailed tests, and statistical significance was set at $p < 0.05$. Discrete variables were assessed using Pearson's chi-square test or Fisher's exact test; continuous variables were assessed using the *t* test for independent samples.

Results

Data were collected from 150 foreign-born patients (73.3% female and 26.7% male) who consulted at the Allergy Department of Hospital Universitario La Paz, Madrid, Spain, with a diagnosis of respiratory allergy, between January 2017 and December 2018. Demographic variables are shown in **table I**. Mean age was 38.38 years. The mean time to onset allergic rhinitis/asthma symptoms after immigration was 8.47 ± 7.7 years. Significant differences in the meantime to onset allergic rhinitis/asthma symptoms after immigration were observed between

Table I *- Demographic variables.*

	Number of patients	Sex (M/F*)	Age (mean)	Mean time (years) until symptoms	Mean length time of residence (years)
According to ethnic group					
Caucasian	48 (32%)	9/39	36.98	6.25	10.52
Arab	11 (7.3%)	3/8	38	9.73	18.73
Black	17 (11.3%)	6/11	41.88	10.35	14.65
Asian	19 (12.7%)	7/12	37	13.42	17.79
Latino	55 (36.7%)	15/40	39.07	7.87	13.78
Total	150	40/110	38.38	8.47	13.71
According to area of origin					
Europe	22 (14.7%)	6/16	40.5	5.82	10
Asia	19 (12.7%)	6/13	35.68	13.42	18.26
Africa	13 (8.7%)	3/10	40.54	11.77	20.46
South America	96 (64%)	25/72	38.14	7.66	12.74
Total	150	40/110	38.38	8.47	13.71

*M: male/F: female.

ethnic groups (p = 0.007), with values ranging from 6.25 years in Caucasian to 13.42 years in Asian. No significant differences were recorded when the time to onset of respiratory symptoms was assessed according to area of origin. The mean time to development of rhinitis and conjunctivitis was 9 ± 6.8 years. The mean time to development of asthma was 7.73 ± 9 years.

Clinical characteristics are presented in **table II**. Surprisingly we found that 17.3% of patients had only allergic asthma without rhinitis, in contrast with the 3.2% reported in another Spanish series (5), while 48.7% had only rhinitis and conjunctivitis. Statistically significant differences in forced vital capacity were found according to the area of origin (p = 0.008), with results ranging from 67% in patients from Africa, to 100% in patients from Europe.

Sensitization against allergens is reported in **table III**. Statistical differences were found for grass pollen (p = 0.005), *Cupressus arizonica* (p = 0.032) and *Olea europaea* (p = 0.049). Sensitization to grass pollen was more common among patients from South America, and sensitization to *C. arizonica* and *O. europaea* were more common among patients from Asia. According to the area of origin, statistically significant differences were found regarding levels of specific IgE to *C. arizonica* (p=0.026) with the highest levels in patients from Asia (mean 24.26 kU/L). Specific IgE levels differed between ethnic groups for *Platanus acerifolia* (p = 0.023), with lower levels in Caucasian patients (mean 1.88 kU/L). No significant differences between ethnic groups or area of origin were detected in other variables, such as comorbidities, allergic background, ESPRINT and ACT scores, and eosinophil count in peripheral blood.

Discussion

As of January 2018, foreign-born residents accounted for 13.4% of the population (893,276 people; (52.16% women and 47.84% men)) of the Community of Madrid. Mean age was 34.5 years. By continent, 41.65% were from Europe (more

Table II *- Clinical characteristics.*

	Atopic dermatitis	Drug allergy	Food allergy	Asthma	Rhinitis and conjunctivitis	ESPRINT (Mean)	ACT NC*	ACT PC**	ACT C***	FVC Mean
According to ethnic group										
Caucasian (n = 48)	6 (12.5%)	6 (12.5%)	3 (6.2%)	26 (54.2%)	39 (81.2%)	3.32	22	9	2	102.3
Arab (n = 11)	3 (27.3%)	0	2 (18.2%)	6 (54.5%)	7 (63.6%)	3.26	3	4	1	96.3
Black (n = 17)	0	5 (29.4%)	3 (17.6%)	9 (52.9%)	14 (82.4%)	2.87	8	0	1	87.9
Asian (n = 19)	0	0	3 (15.2%)	**11 (57.9%)**	17 (89.5%)	2.78	7	4	1	89.7
Latino (n = 55)	6 (10.9%)	6 (10.9%)	6 (10.9%)	27 (49.1%)	47 (85.5%)	3.66	27	4	3	97.8
Total (n = 150)	15 (10%)	17 (11.3%)	17 (11.3%)	79 (52.7%)	124 (82.7%)	3.32	67	21	8	97.4
According to area of origin										
Europe (n = 22)	1 (4.5%)	4 (18.2%)	2 (9.1%)	11 (50%)	19 (86.4%)	2.68	7	6	1	109.4
Asia (n = 19)	18 (94.7%)	0	3 (15.8%)	10 (52.6%)	17 (89.5%)	2.86	7	3	1	88.9
Africa (n = 13)	10 (76.9%)	0	2 (15.4%)	**8 (61.5%)**	8 (61.5%)	3.33	4	4	1	90.0
South America (n = 96)	86 (86.9%)	13 (13.5%)	10 (10.4%)	50 (52.1%)	80 (83.3%)	3.56	49	8	5	96.8
Total (n = 150)	135 (90%)	17 (11.3%)	17 (11.3%)	79 (52.7%)	124 (82.7%)	3.32	67	21	8	97.4

* Not controlled. ** Partially controlled. *** Controlled.

Table III *- Sensitization against allergens.*

	Grass pollen	*Olea europaea*	*Platanus acerifolia*	*Cupressus Arizonica*	Mold	Cat	Dog	Mites
According to ethnic group								
Caucasian (n = 48)	**32 (66.7%)**	26 (54.2%)	17 (35.4%)	26 (54.2%)	1 (2.1%)	15 (31.2%)	11 (22.9%)	18 (37.5%)
Arab (n = 11)	**8 (72.7%)**	6 (54.5%)	4 (36.4%)	8 (72.7%)	0 (0%)	2 (18.2%)	2 (18.2%)	5 (45.5%)
Black (n = 17)	**13 (81.2%)**	10 (62.5%)	5 (31.2%)	7 (43.8%)	0 (0%)	6 (37.5%)	4 (25%)	7 (43.8%)
Asian (n = 19)	14 (73.7%)	14 (73.7%)	10 (52.6%)	**16 (84.2%)**	1 (5.3%)	4 (21.1%)	5 (26.3%)	7 (36.8%)
Latino (n = 55)	**45 (81.8%)**	32 (58.2%)	24 (43.6%)	32 (58.2%)	2 (3.6%)	12 (21.8%)	13 (23.6%)	26 (47.3%)
Total (n = 150)	112 (75.2%)	88 (59.1%)	60 (40.3%)	89 (59.7%)	4 (2.7%)	39 (26.2%)	35 (23.5%)	63 (42.3%)
According to area of origin								
Europe (n=22)	**10 (45.5%)**	8 (36.4%)	9 (40.9%)	**10 (45.5%)**	1 (4.5%)	9 (40.9%)	7 (31.8%)	8 (36.4%)
Asia (n = 19)	14 (73.7%)	15 (78.9%)	10 (52.6%)	**16 (84.2%)**	1 (5.3%)	4 (21.1%)	5 (26.3%)	6 (31.6%)
Africa (n = 13)	**10 (76.9%)**	8 (61.5%)	5 (38.5%)	**10 (76.9%)**	0 (0%)	3 (23.1%)	3 (23.1%)	5 (38.5%)
South America (n = 96)	**78 (82.1%)**	57 (60%)	36 (37.9%)	53 (55.8%)	2 (2.1%)	23 (24.2%)	20 (21.1%)	44 (46.3%)
Total (n = 150)	**112 (75.2%)**	88 (59.1%)	60 (40.3%)	89 (59.7%)	4 (2.7%)	39 (26.2%)	35 (23.5%)	63 (42.3%)

than a half, from Eastern Europe), 12.63% were from Africa (mainly Morocco), 34.36%, were from America (mainly South America), and 11.25% were from Asia (60% from China) (14). Many publications report a probable relationship between time to onset of allergic symptoms and demographic variables such as length of residence in a new place and age on arrival (15, 16). These results are similar to ours in adults, who become sensitized to the same allergens as the local population after a mean of 8.47 years, although this figure is lower in other reports (4.5 years according to Álava *et al.* in the same region of Spain (17)). In our population, the time to onset of allergic respiratory symptoms was lower in the Caucasian group and higher in the Asian group; Domínguez-Ortega *et al.* (9) reported this time to be lower for Latinos (possibly because of the seasonal bias).

The predominance of respiratory allergy in foreign-born women (or at least, the predominance of consultation) is observed in other studies (9) as well as ours.

Even though we found a statistically significant predominance of sensitization to grass pollen in patients from South America and to *C. arizonica* and *O. europaea* pollen in patients from Asia, our data show that, the allergy sensitization profile in the foreign-born population we studied was similar to that of the local population after some time living in Madrid (9, 18). Previous reports on immigrant sensitization profiles show contradictory results. Domínguez-Ortega *et al.* (9) found significant differences in the sensitization pattern of respiratory allergy in immigrants living in Madrid. Studies in Italy also report differences in the sensitization profile, with a higher risk of sensitization to grass in patients from Sub-Saharan Africa and South America and a higher risk of sensitization to house dust mites in immigrants from South Asia (8). A relevant difference between native residents and immigrants, as well as relevant differences between immigrant themselves, was reported by Ciprandi *et al.* (19) in Italy, with more frequent sensitization to mites in people from South America, more frequent sensitization to *Parietaria* and birch in people from North Africa and South America, and more frequent sensitization to grass in people from South America. In our study, sensitization to grass pollen was the most frequent type. According to Belver *et al.*(18), the second most common type of sensitization was to *O. europaea* pollen. These data coincide partially with ours, although sensitization to *C. arizonica* seems to be as common as sensitization to *O. europaea*. *P. acerifolia* and mites was also considerable in the present study population. Our study may be subject to bias resulting from de-

mographic and clinical factors. Most patients were from Tetuán neighborhood of Madrid, which is near our hospital and home to, large percentage of people from South America, thus explaining differences with other series reported in the Community of Madrid. The results could be affected by other sources of bias, such as the type of patient who consults most frequently, or how people view the severity of their symtomps. However, since study data were collected prospectively over 13 months, they cover all pollination periods. Results from cross-sectional studies (9) could be affected by seasonality.

To our knowledge, ours is the first study to report specific IgE levels, which were higher for *P. acerifolia* in Latinos (10.65 kU/L vs a mean of 7.29 kU/L), lower for *P. acerifolia* in Caucasians (mean 7.29 kU/L), and higher for *C. arizonica* in patients from Asia (24.26 kU/L vs mean of 11.13). A possible explanation for this difference could be previous exposure of Asians to other Cupressaceae such as *Juniperus chinensis* in their countries of origin. The statistically significant differences in forced vital capacity according to the area of origin (100% in patients from Europe, 78% in patients from Asia, 67% in those from Africa, and 93% in those from South America), could be due in part to the reference values used (20). Most equations used in spirometry can be applied equally to different ethnic groups, although additional data are needed from other areas (*e.g.* Indian subcontinent, Africa, and Arab, Polynesian, and Latin American countries), to improve these equations in the future (21).

Symptoms to olive pollen could be the consequence of sensitization to cross-reactive allergens from other sources such as Fra e 1, a major allergen in Fraxinus allergy, or group 11 in grass pollen. However, that specific analysis has not been performed in this real life study.

17/150 patients had food allergy. Only 1 of them had LTP allergy so there is not a probable bias in *P. acerifolia* sensitization. 7 patients had Oral Allergy Syndrome (OAS) with profilin implication. According to our results foreign-born population acquired a mostly genuine sensitivity after some years living in Spain in contact with new allergens.

There were no statistically significant differences in the prevalence of allergic asthma or rhinitis, and/or conjunctivitis between ethnic groups or according to area of origin. Surprisingly we found that 17.3% of patients had only allergic asthma without rhinitis, in contrast to other Spanish series, in which only 3.2% had allergic asthma without rhinitis (5). Rhinitis and conjunctivitis without asthma was recorded in 48.7%. No significant differences were found in comorbidities, allergic background, ESPRINT and ACT scores and blood eosinophil count. However, our results may have been affected by the small size of some of the groups (only 11 Asian and 13 African patients). Moreover, not taking into account other factors such as socioeconomic status and occupation, might also influence the results.

To conclude, sensitization and development of symptom of allergic disease in foreign-born patients seem to be influenced by environmental factors such as length of residence in the new country and prevalence of local allergen. Foreign-born residents have the same sensitization profile as the native population (9, 18). Nevertheless, we did identify differences in the sensitization profile according to ethnic group and area of origin, thus highlighting the complexity of sensitization, in which both genetics and environmental factors interact. Prospective studies with a larger sample are needed to determine whether significantly different patterns can be identified in foreign-born patients.

Conflict of interests

The authors declare that they have no conflict of interests.

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Association between the density and type of trees around urban schools and exhaled nitric oxide levels in schoolchildren

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Summary
Greenspaces in school's neighbourhood represent an important environment to promote healthy development. The aim of this study was to assess the association between the density and type of trees around schools and exhaled Nitric Oxide (NO) levels in schoolchildren. Data on 845 children from 20 primary schools in Porto was analysed. Airway inflammation was assessed by measuring exhaled NO level. The density and type of trees were quantified within a 500 m buffer around schools. Associations were estimated using mixed-effect models. A significant association was observed between non-tree covered areas around schools and exhaled NO levels in schoolchildren (β = - 1.42, 95% CI - 2.84, - 0.001). Our results suggested that the presence of trees in school neighbourhoods may play a role in the biological mechanisms underlying the complex links between environment and airway inflammation.

Introduction

Growing urbanization is globally changing urban-natural environment, biodiversity as well as human lifestyles, which may have important public health implications (1, 2). Although urban life offers a greater access to community services, it is also associated with increased exposure to air pollution and loss of natural environments (3, 4). With hasty global urbanization, there is an increasing interest in understanding how urban settings and environment affect children's health.

Urban green spaces not only provide balance for ecosystems but can also act as a buffer against exposure to air pollution, by removing pollutants from the atmosphere (18, 19). Pollutants may be removed from the atmosphere through wet and dry deposition on the tree surface and/or by stomatal adsorption and absorption processes (20). Knowledge on how greenspaces affect human health is crucial in addressing the increasing prevalence of asthma and allergies worldwide and to direct urban and community planning for a healthy environment (21, 22). Previous studies have examined the effect of air pollution ex-

posure on exhaled Nitric Oxide (NO) levels, as a non-invasive measure of airway inflammation (23), but no studies evaluate the role of greenspaces on exhaled NO in children. Thus, the aim of this study was to assess the association between the density and type of trees around schools and exhaled NO levels in schoolchildren.

Materials and methods

The present study included participants from a cross-sectional study assembled in 20 primary schools in Porto, Portugal, corresponding to a total of 71 assessed classrooms (24). The University Health Ethics Committee approved the study, and informed consent was obtained from the children's legal guardians. All research was performed in accordance with the Declaration of Helsinki.

Physical and clinical assessment

A self-administered ISAAC-based questionnaire (25) was filled out by children's parents covering information on social, demographic and behavioural characteristics and questions regarding the respiratory/allergic health of the children. A physical and clinical assessment, including height, weight, spirometry with bronchodilation, exhaled NO, and Skin-Prick-Tests (SPTs) were performed by trained health professionals (24).
Lung function and airway reversibility were assessed by spirometry according to ATS/ERS guidelines (26) using a portable spirometer (MIR Spiro bank, A23-04003237) before and 15 minutes after the inhalation of 400 µg of salbutamol. The definition of asthma was based on clinical and functional criteria: self-report of asthma diagnosed by a physician (medical diagnosis), with reported asthma symptoms over the past 12 months and/or at least a 12% and over 200 mL increase in FEV1 (Forced Expiratory Volume in 1 second) after bronchodilation (27).
Airway inflammation was assessed by measuring the fractional exhaled NO level using a NO breath analyzer (Bedfont Scientific, Ltd.) in accordance with the ATS guidelines (28).
SPTs were performed on children forearm using a QuickTest™ applicator (Hall Allergy) and allergen extracts of house dust mite, mix of weeds, mix of grasses, cat dander, dog dander and *Alternaria alternata*, a negative (extracts diluent) and a positive control (histamine at 10 mg/mL) (Hall Allergy, Netherlands). Results were read 20 minutes afterwards and atopy was defined by a positive SPT to at least one of the allergens.

Density and type of trees

Dominant leaf type (broadleaved/coniferous) and tree cover density (percentage of tree coverage, 0 to 100%) at 20 m spatial resolution were obtained from the 2015 High Resolution Layer (HRL) Forest products, which can be obtained from the Copernicus Land Monitoring Service (CLMS), coordinated by the European Environment Agency (EEA) (29).
A circular buffer of 500 metres around each participant's primary school address was created (**figures 1, 2**) in ArcGIS 10.5.1 (Environmental Systems Research Institute, ESRI, Redlands, CA, USA). This buffer has been previously considered by Paciência *et al.* (9) since it is considered a reasonable walking distance for children as described by Brownson *et al.* (30).
Then, each buffer was overlaid with the maps depicting the dominant leaf type and tree cover density to determine the tree cover density (0-100%) and the percentage of area covered by each dominant leaf type in the school surroundings. Overlay operations were performed using QGIS 3.8. Weighted average of tree cover density and the total area of broadleaves, coniferous and non-tree covered area were calculated for each school's neighbourhood to determine the percentage and type of trees.

Study participants

In total, 1602 children (7-12 years old), all in the 3rd and/or 4th grades, were invited to participate. Among them, 686 did not return the signed informed consent form (participation rate of 57%), and 58 refused to perform clinical tests.

Data analysis

Distribution of continuous variables was analysed for normality. Whenever non-Gaussian distributions were observed, non-parametric tests were performed for inferential statistics. The Kruskal-Wallis test was used to compare variables between girls and boys. Significant differences were defined according to an α-value of 5% ($p < 0.05$). Mixed-effect models with a random effect at school-level were used to measure the influence of density and type of trees around schools on exhaled NO levels in children. The Intraclass Correlation Coefficient (ICC) was used to quantify the proportion of the total variance that is at the school's neighbourhood level. The following confounders were considered in the adjusted models: age, sex, asthma, atopy, parental education level and exposure to tobacco smoke at home. Associations were expressed as standardized beta coefficients, β, and 95% CI. Statistical analysis was performed using R.

Results

Among the 858 included children, 13 were excluded due to poor quality lung function test data. Hence, this study was based on data from 845 children (49.2% girls) (**table I**). The median (25^{th}-75^{th}) levels of exhaled NO was 11.0 (6.0-20.0) ppb, being higher among boys (12.0 (6.0-21.0) *vs* 10.0 (6.0-17.5), $p = 0.004$). The prevalence of asthma, atopy and rhinitis

Figure 1 *- Percentage of tree cover around each primary school. Each school was represented by a point and a circular buffer of 500 metres.*

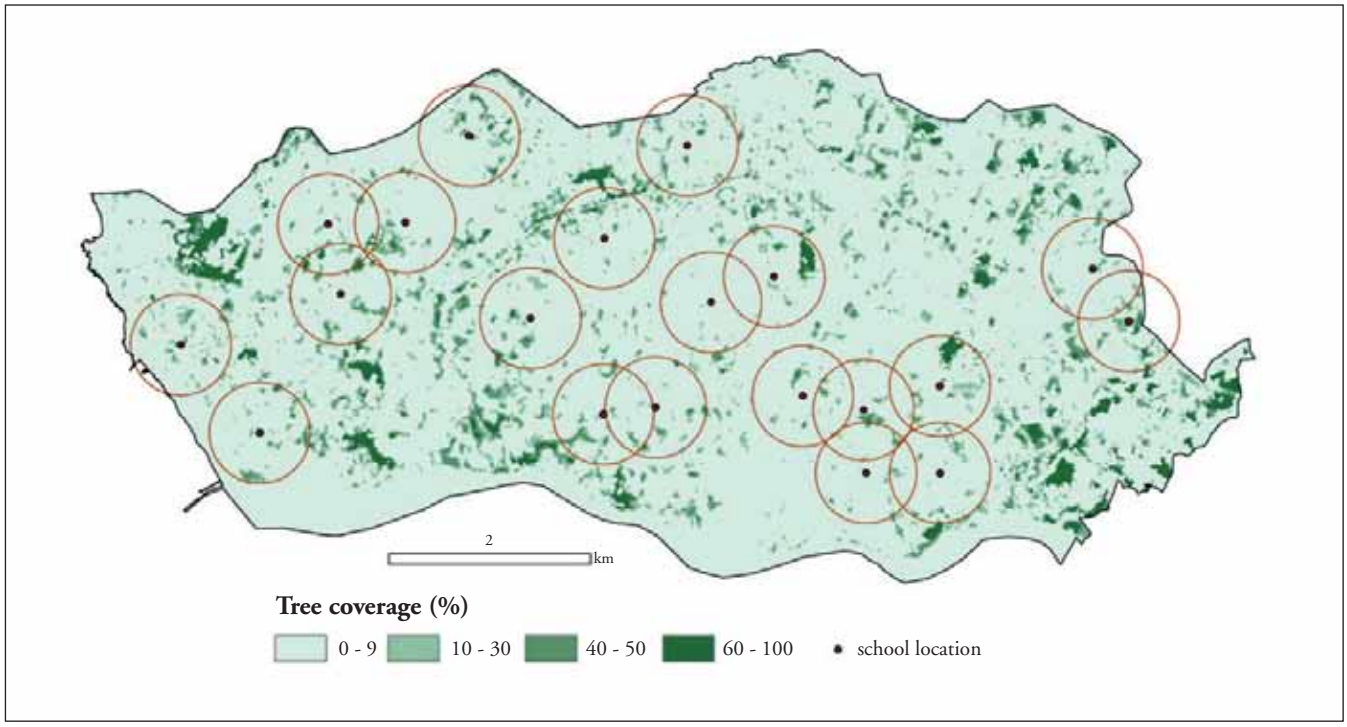
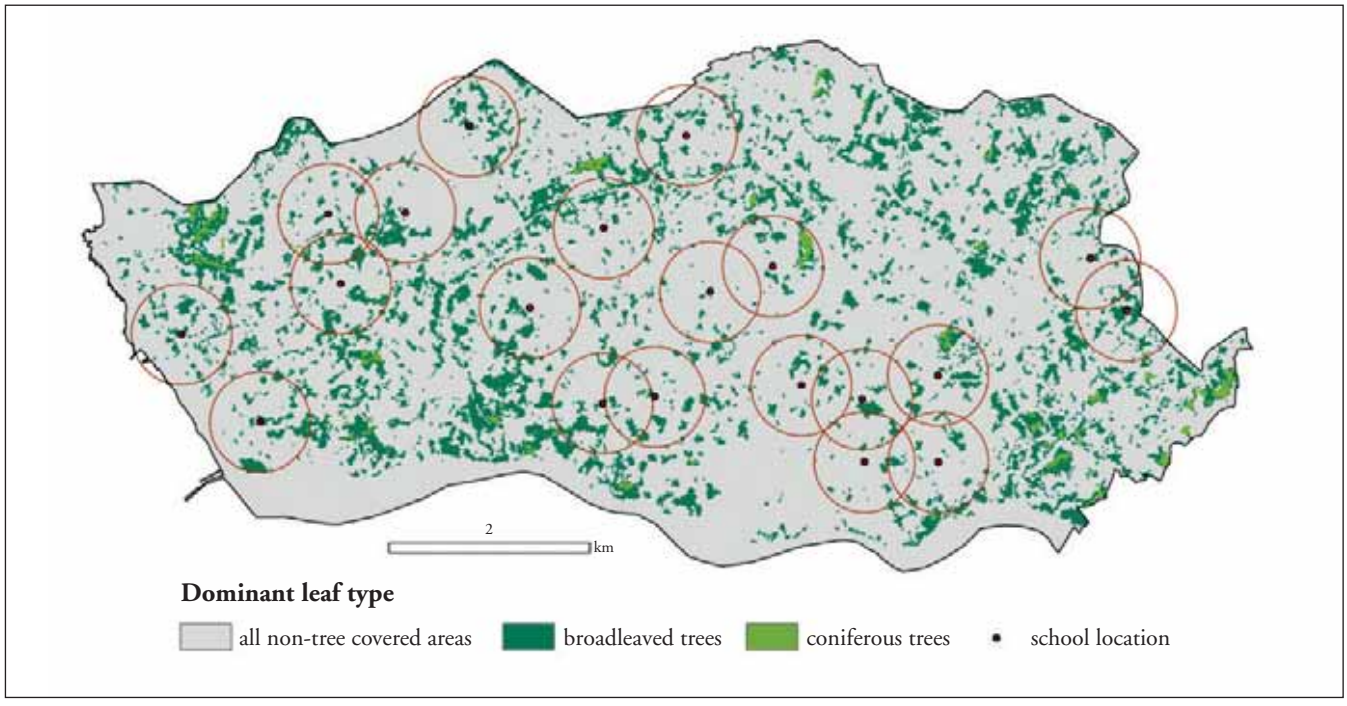


Figure 2 *- Dominant leaf type around each primary school. Each school was represented by a point and a circular buffer of 500 metres.*



was 9.5%, 35.4%, and 13.4%, respectively. The prevalence of overweight or obesity was nearly 30% (**table I**).

The surrounding density and type of trees is presented in **table II**. The percentage of tree cover ranged from 16% to 37%, and the median area covered by coniferous and broadleaves was 10.8 ha and 0.12 ha, respectively. The median (25^{th}-75^{th} percentile) of non-tree covered area was 64.6 ha (58.5-66.7 ha) (**table II**). Positive Pearson's correlations were found between tree cover density and type of trees (rho = 0.430 (broadleaves) and 0.751 (coniferous), $p < 0.05$). The density of tree cover and the distribution of broadleaves and coniferous was significantly different between schools ($p < 0.05$).

A significant association was found between non-tree covered areas around school and the levels of exhaled NO in schoolchildren (model 2: β = - 1.42, 95% CI - 2.84, - 0.001). An increment of 10 m^2 in non-tree covered areas around schools was associated with a decrease of 1.4 ppb in exhaled nitric oxide levels. The association was similar but non-significant between the presence of broadleaves (β = - 0.14, 95% CI - 0.49, 0.22), coniferous (β = - 1.16, 95% CI - 3.09, 0.76) and tree cover density (β = - 0.01, 95% CI - 0.04, 0.01) and exhaled NO levels in children (**table III**).

The ICC revealed that approximately 4.0% of the variance in the levels of exhaled NO is at neighborhood-level. The tree cover density, non-tree covered areas, broadleaves and coniferous in school's neighborhoods explained 3.84%, 9.10%, - 7.76% and - 3.82% of the levels of exhaled NO, respectively.

Discussion

Our study shown how density and type of trees surrounding primary schools may influence the levels of exhaled NO in children. After adjustment, non-tree covered areas around primary schools were inversely associated with children's exhaled NO, independently of asthma and allergy status, suggesting that the presence of trees in school neighbourhood may affect the levels of exhaled NO in both healthy and susceptible children.

The present study has some limitations. The cross-sectional design does not allow the determination of causal associations and exposure-dose assumptions. Furthermore, we did not address the

Table I - *Characteristics of the participants.*

Characteristics	**Total (n = 845)**	**Girls (n = 416)**	**Boys (n = 429)**
Age [years (mean ± SD)]	9.0 (8.0-9.0)	9.0 (8.0-9.0)	9.0 (8.0-9.0)
Parental education [n (%)]			
≤ 9 years	219 (32.1)	97 (29.6)	122 (34.5)
10-12 years	201 (29.5)	103 (31.4)	98 (27.7)
≥ 13 years	262 (38.4))	128 (39.0)	134 (37.9)
Exposure to smoke at home [n (%)]			
Never	588 (76.4)	293 (76.5)	295 (76.2)
Daily	72 (9.4)	32 (8.4)	40 (10.3)
1-4 times per week	20 (2.6)	8 (2.1)	12 (3.1)
1-3 times per month	90 (11.7)	50 (13.1)	40 (10.3)
Exhaled NO [ppb (median (25^{th}-75^{th} percentile)]	11.0 (6.0-20.0)	**10.0 (6.0-17.5)**	**12.0 (6.0-21.0)**
Asthma [n (%)] ǂ	80 (9.5)	**49 (11.8)**	**31 (7.2)**
Atopy [n (%)] ǂ	296 (35.4)	145 (35.3)	151 (35.6)
Rhinitis [n (%)]	101 (13.4)	**40 (10.8)**	**61 (16.0)**
BMI, CDC [n (%)]			
Underweight	39 (4.6)	21 (5.0)	18 (4.2)
Normal weight	575 (68.0)	281 (67.5)	194 (68.5)
Overweight	128 (15.1)	67 (16.1)	61 (14.2)
Obese	103 (12.2)	47 (11.3)	56 (13.1)

BMI: Body Mass Index; CDC: US Centres for Disease Control; Bold corresponds to significant differences (p value < 0.05).

quality and type of vegetation or biodiversity. Nevertheless, our analyses were based on the objective measure of tree cover density developed by the European Environmental Agency, allowing a comparison across studies, a better understanding of effects of specific type of trees and the complexities in the interactions between greenspaces and exhaled NO levels, and avoiding bias related to participants' perception of their neighbourhoods. Our results are also limited by low Intraclass Correlation Coefficients (ICCs) generated by the variables of the multilevel analysis. Further, the between-neighbourhood variance was reduced with the inclusion of the individual-level variables of age, sex, asthma, atopy, parental education level and exposure to tobacco smoke at home. However, even low ICCs may have an important impact on levels of exhaled NO in children (31), being important pre-

Table II *- Distribution of density and type of trees around schools.*

	Median (25th-75th percentile)	**Min-Max**
Tree cover density (%)	21.0 (20.0-27.0)	16.0-37.0
Non-tree covered area (ha)	64.5 (58.5-66.7)	40.0-69.7
Broadleaves (ha)	10.1 (7.48-12.2)	2.56-19.3
Coniferous (ha)	0.12 (0.04-0.48)	0-2.80

Table III *- Multilevel model analysis of the association between density and type of trees and exhaled NO level.*

Characteristics	**Total (n = 845)**	**Girls (n = 416)**	**Boys (n = 429)**
Tree cover density			
β (95% CI)	---	- 0.01 (- 0.02, 0.001)	- 0.01 (- 0.04, 0.01)
ICC (%)	3.89	3.84	6.11
Variance	0.03	0.03	0.05
Variance explained (%)	Reference	1.19	- 45.6
Non-tree covered area			
β (95% CI)	---	- 0.89 (- 2.16, 0.38)	**- 1.42 (- 2.84, - 0.001)**
ICC (%)	3.50	3.55	4.93
Variance	0.03	0.03	0.04
Variance explained (%)	Reference	9.10	- 16.0
Broadleaves			
β (95% CI)	---	- 0.04 (- 0.20, 0.12)	- 0.14 (- 0.49, 0.22)
ICC (%)	3.80	4.18	6.78
Variance	0.03	0.04	0.05
Variance explained (%)	Reference	- 7.76	- 62.6
Coniferous			
β (95% CI)	---	- 0.51 (- 1.33, 0.32)	- 1.16 (- 3.09, 0.76)
ICC (%)	3.60	4.03	6.32
Variance	0.03	0.03	0.05
Variance explained (%)	Reference	- 3.82	- 50.9

ICC: Intraclass Correlation Coefficient; β: beta regression coefficients; 95% CI: 95% Confidence Intervals.
Model 0: is null model, baseline model without any exposure variable; Model 1: only included the variable of exposure; Model 2a: adjusted for age, sex, asthma, atopy, parental education level and exposure to tobacco smoke at home.

dictors of health outcomes and compatible with important policy effects of school's neighbourhood characteristics (32). Finally, other confounders related to exposures at school and home, namely air pollution, was not measured in the study. However, data on asthma, atopy, parental education, and exposure to tobacco smoke were considered as potential confounding factors.

The chosen buffer size can also affect the results, however the 500 m buffer is supported by Browning *et al*. (33) and Brownson *et al*. (30), and has been associated with a less prone to exposure misclassification (34).

Our study has also important strengths. To our knowledge, this is the first study evaluating the effect of density and type of trees in schools' neighbourhoods on levels of exhaled NO in schoolchildren. Additionally, we performed a comprehensive clinical assessment with a large number of participants, while controlling for a number of potentially important confounders. The Global Initiative for Asthma (GINA) guidelines define a significant bronchodilator response as an increase in FEV1 ≥ 12%, in children (35). However, we considered an increase in FEV1 ≥ 12% and/or 200 ml as recommended by the NICE guideline committee members (36) as being a more appropriate threshold as evidence of a positive test in response to a standard dose of bronchodilator. In addition, American Thoracic Society recommendations define a significant bronchodilator response as an increase in FEV1 ≥ 12% and/or 200 ml in both adults and children (37). The effect of trees on schools' neighbourhoods was evaluated using a robust statistical tool that allowed a multilevel approach, considering the complex relationship among the different levels of variables. Nevertheless, it will be important to assess the effect of long-term exposure to school neighbourhoods to understand the extent of health effects.

Nitric oxide is present in exhaled breath and plays a number of key roles in lung physiology, including as a vasodilator, bronchodilator, and inflammatory mediator (38), raising the question of whether exhaled NO can be used as a biomarker of the adverse effect of air pollution on the airways. In addition, Tarantini *et al*. (39) shown that exposure to air pollutants, including particulate matter ($PM_{2.5}$), may impacts iNOS (induced NOS) promoter methylation. Nitric oxide synthase catalyzes the generation of nitric oxide and three isoforms has been identified – iNOs, eNOS (endothelial NOS) and nNOS (neural NOS). iNOS has been associated to increased levels of nitric oxide, being also responsible for the higher levels of exhaled NO (40). Similar to findings of Tarantini *et al*. (39), a study including 940 children (6-11 years of age) suggested that the effect of air pollutants on exhaled NO levels may be mediated by a higher iNOS expression due to a lower methylation in iNOS promoter (41). In fact, Salam *et al*. (41) reported that exposure to higher concentrations of $PM_{2.5}$ and lower iNOS methylation was related to higher iNOS expression and consequently to higher levels of exhaled NO in children.

Our results suggested that exhaled NO in children may be a sensitive marker for respiratory effects related to school neighborhood. Our results showed that exhaled NO in schoolchildren may decrease with increased percentage of non-tree covered area, tree cover density, broadleaves and coniferous. Green spaces in school neighborhoods can have beneficial effects on air pollution through absorption, providing physical barriers against emission sources, or by limiting the overall area available to sources of pollutants, such traffic or industry (42, 43). In fact, in our study the non-tree covered areas were inversely correlated with the presence of fast transit roads and industrial areas surrounding schools, supporting a decrease of pollutants concentration and consequently on the levels of exhaled NO. Mechanistically, a decrease of air pollutants may associate with a lower activation of Transient Receptor Potential channels (TRP), such as TRPA1 and TRPV1, reducing the production of inflammatory cytokines and nitric oxide (44).

Previous studies have also reported associations between exposure to air pollution, especially in children, and increased levels of exhaled NO (45, 46). Short-term exposure to higher concentrations of particulate matter ($PM_{2.5}$ and PM_{10}) and ozone were associated with airway inflammation independent of asthma and allergy status (45). The negative impact of exposure to urban environment has also been further reinforced by Berhane *et al*. (46), in which exposure to urban air, particularly to nitric dioxide and $PM_{2.5}$, was positively associated with changes in exhaled NO levels in children. The levels of exhaled NO observed in our study were similar to mean values found by Berhane and collegues in 2011 (11.02 ppb in girls and 11.25 ppb in boys) (45) and 2014 (14.8 ppb in girls and 16.6 ppb in boys) (46). Although Berhane *et al*. (45) underline that levels of air pollution may have the potential to increase the levels of exhaled NO in both healthy and susceptible children, heterogeneity in results may be increased by the different levels of exposure.

Our results suggested that density and type of trees in school neighbourhoods may play a role in the biological mechanisms underlying the complex links between environment and airway inflammation. However, the absolute changes in NO that we observed may be considered small in terms of their possible clinical impact. Additional studies evaluating both the density, type of trees and levels of air pollutants, as well as the interaction with greenspaces and pollution in relation to airway inflammation, should be conducted in order to better understand the mechanisms behind the effects associated with urban environment. Our results suggest that the density and type of trees in school neighbourhood may have an impact on exhaled NO in children. Ultimately, this study supports the concept that urban greenspaces and the presence of trees in school neighbourhoods may be associated with respiratory health in the long-term, contributing to the implementation of future urban planning policies and practices that may promote a healthy lifestyle and reconnection with nature.

Conflict of interests

The authors declare that they have no conflict of interests.

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Drug use and abuse and the risk of adverse events in soccer players: results from a survey in Italian second league players

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Key words
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Summary

Objective. *Drug use in athletes has been frequently investigated in the last three decades, especially regarding its misuse for doping. However, little is known about the use of permitted drugs for medical purposes and less studies have investigated the relationship between adverse drugs reactions (ADRs) and sports.* **Methods.** *An observational cross-sectional investigation analyzing a group of second league soccer players (the second-highest division in Italy) was performed. Anamnestic and physical examinations as well as a validated questionnaire (AQUA©) were performed in a group of 378 Italian second league soccer players.* **Results.** *Most players (91.8%) reported the use of NSAIDs in the previous year, and one third of them were regular users. Analgesics were used in 64% of the players, while 52.1% had taken antibiotics in the previous year. 29.20% of players used intraarticular treatments in the previous year. In 7,4% of players, an ADRs was reported: 3,47% reacted to NSAIDs, 2,6% to antibiotics, 1,05% to analgesics and 1 of them to supplements. For intra-articular injections, only 2 players experienced ADRs. One quarter of players experienced reactions as urticaria-angioedema syndrome or more severe conditions as bronchospasm or anaphylaxis.* **Conclusions.** *This study shows that drug misuse/abuse in soccer is a real matter of debate, especially with regards to NSAIDs, exposing athletes to predictable and/or unpredictable risks for their health.*

Introduction

In the last three decades, drug use in sports has been frequently investigated (1). The use of drugs not for medical purposes but as performance enhancers is widely known as "doping". The first official ban of performance enhancing drugs dates back to 1928 as the usage of "stimulating substances" was forbidden by the International Amateur Athletic Federation (2). The World Anti-Doping Agency has prohibited through the years the use of many substances which result in gaining advantage in performance, a risk for health, or in violating the "spirit of sport" (3). The Doping practice has "ancient" roots: the employment of performance-enhancing remedies goes back to Ancient Olympics (4). Several studies have been produced about misuse of drugs for doping in sports (5) while very little is known about the use of permitted drugs for medical purposes (6). A retrospective survey about the use of medication during FIFA World Cup 2014 in Brazil has shown that amongst top level players 67% took medications:

54.2% of them used Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) while analgesics were taken by 12.6% and β-2 agonists by 0.5%. Before each match, on average 0.8 medications per player were used (7). In Italy, an epidemiological study in 2007 showed that the regular assumption of permitted drugs has a high rate among professional soccer players. In the previous year, 92.6% of players assumed NSAIDs, 86.1% were current users, and 36% of them used analgesics. Moreover, 82.8% of the players used supplements, 28% of which were vitamins (8). No other investigations on this topic are available in the literature. Indeed, a limited range of studies has investigated adverse the rate of Adverse Drugs Reactions (ADRs) in agonistic sport athletes.

The World Health Organization (WHO) classifies hypersensitivity reactions to NSAIDs in type B adverse drug reaction (unpredictable and occurring in susceptible individuals) which differs from those predictable, based on pharmacological mechanisms and occurring in all individuals if a sufficient dose is applied (type A reactions) (9).

Based on the most recent EAACI/WAO nomenclature, hypersensitivity reactions to NSAIDs are further divided into 1) allergic-immunologically mediated (non-cross-reactive) and 2) non allergic-non immunologically mediated (cross-reactive) hypersensitivity reactions (9).

Stevenson *et al.* originally classified hypersensitivity reactions to NSAIDs accordingly to the clinical manifestation, the occurrence of an underlying disease, and a cross-reactivity with other inhibitors of the Cyclooxygenase (COX)-1 (10). A not immunological reaction is responsible of the hypersensitive to chemically nonrelated NSAIDs, sharing the common property of COX-1 enzyme inhibition and is called the 'cross-reactive' type of NSAIDs hypersensitivity. On the other hand, hypersensitivity symptoms that occur only after the ingestion of a single, specific NSAID (or belonging to the same chemical group), are considered immunologically mediated and defined as "allergic hypersensitivity reactions". The putative immunological (IgE or T-cell mediated) or nonimmunological (cross-reactive) mechanism is defined on the basis of the patient reaction and/or the timing (11).

According to WAO, drug allergy is a relatively uncommon event, which represents less than 10% of all ADRs. It is responsible for 1% to 2% of all hospital admissions and 3% to 5% of hospitalized patients (12), although the true incidence of drug allergy in the community is still unknown. There are many risk factors for drug allergy, and they may be both drug related, and host related. Among the drug related factors, the degree of exposure in terms of dose, duration, and frequency of assumption should be considered (13). The aim of our work was to establish the prevalence of use of permitted drugs in a cohort of Italian professional soccer players as well as to investigate on its related ADRs rate.

Study design and subjects

The study was designed as an observational cross-sectional investigation analyzing a group of second league soccer players (the second-highest division in Italy). Players were consecutively enrolled during medical checkup performed training sessions. No particular criteria of inclusion and/or exclusion were chosen for the study enrollment. All subjects were enrolled from July 2015 to May 2016: each subject agreed to participate in the study and consent form was obtained.

Materials and methods

On a group of 378 Italian second league soccer players, anamnestic and physical examinations were performed with specific questionnaires being administered.

Anamnestic evaluation was focused on personal and family history of chronic diseases and on physical activities habits. A validated self-report Allergy Questionnaire for Athletes (AQUA©) (14), with additional questions pertaining to training history and drug use habits, translated into the 5 main European languages (English, Italian, French, Spanish and German) was fulfilled by players. It included 4 different sections: 1) identity – including sociodemographic data such as gender, age and place of birth; 2) physical activity history – frequency in hours per week of training activity, numbers of years regarding the competitive practice and injuries occurring in career and in last year; 3) pharmacological anamnesis – investigating details on the therapeutic schemes used for traumas and for other common pathologies, players' own use of drugs or about recreational drugs; 4) ADRs – including details about the drug taken, type of reaction (localized or systemic reaction).

Continuous variables are presented as mean (standard deviation), and categorical variables are shown as frequencies and percentages. The independent contribution of several factors (role and age of the player, weekly hours of training, number of training sessions, body mass index) on permitted drug use was assessed using a multivariate general linear model.

Results

The characteristics of the study population (378 Italian second league soccer players) are shown in **table I**: they were all male individuals with a mean age of 24.8 (± 5.4) years and a mean sport duration of activity of 12.1 years. The mean age at the beginning of professional activity was 10.4 years (range 4.0-18.0 years). The amount of physical activity usually performed was > 9 hours per week. About sport injures, 84.8% of players experienced a major injury at least once throughout the whole career: only in 38% the injury occurred during sport activity in the previous 12 months.

Table I *- Characteristics of Italian Serie B football players.*

	Italian Serie B football players group
Age, years ± SD	24.8 ± 5.4
Gender, n (%)	
Male	378 (100%)
Race	
African American	17 (4.5%)
Caucasian	326 (86.2%)
Hispanic-Latino	35 (9.3%)
BMI ± SD Kg/m^2	22.0 ± 1.5
MDSA, years ± SD	12.2 ± 5.1
Hours/week n (%)	
< 3 hrs	0 (0%)
3-7 hrs	11 (2.9%)
> 7 hrs	367 (97.1%)

Muscle, tendon, ligament and meniscus injuries were found in 62.5%, 21.4%, 28.6%, and 24.3%, respectively. In 55.7% of injuried players, muscular lesions were experienced only once in their career and in 21% in the previous year. Tendon lesions occurred in 74% once in the sport career, in 26% more than once through the years and 9% in the previous year. Ligament injuries occurred in 65.2% of players once throughout career, in 34.8% more than once and in 12% in the previous year. Meniscus damage was experienced in 66.7% only once, while more than once in 33.3% with 15% being affected in the previous year (**table II**). Most players (91%) reported the use of NSAIDs in the previous year, and about one third of them were regular users, defined as > 30 days/year consumers (**table III**). Analgesics were used in 64% of the players, while 52.1% had taken antibiotics in the previous year. The drugs used in the previous year are summarised in table 3: 33,7% referred NSAIDs use lasting more than 30 days in the whole year, while 75 players (19.85% of the whole study population) were currently using analgesics. Almost all players reported the use of supplements (98%) and among users on a daily basis (80%), 25% of subjects used supplements more than once a day. Out of 110 players (29.2%) using intraarticular treatments 34 (31%) used platelet gel at least once and 15 of them more than once in the previous year. In total, hyaluronic acid was administered to 84 players (22.3%) and in 39 of them in more than one course of treatment. 2 % of players treated with steroidal intraarticular therapy and one third of them underwent the treatment more than once in the previous year. Other drugs (*i.e.*, anaesthetics) were administered to 2.8% of the players (**table III**).

Table II *- Injuries in soccer players.*

Type of injuries n (%)	**Yes**
Sport injuries	321 (84.8%)
Muscle injury	201 (62.5%)
Frequency:	
1 time	112 (55.7%)
> 1 time	89 (44.3%)
Tendon injury	69 (21.4%)
Frequency:	
1 time	51 (74%)
> 1 time	18 (26%)
Ligament injury	92 (28.6%)
Frequency:	
1 time	60 (65.2%)
> 1 time	32 (34.8%)
Meniscus injury	78 (24.3%)
Frequency:	
1 time	52 (66.7%)
> 1 time	26 (33.3%)
Injuries occurred during the previous year	
Tot	122 (38 %)
Muscles	67 (21 %)
Tendon	29 (9 %)
Ligament	38 (12 %)
Meniscus	48 (15 %)
Other injuries	
Upper limb	48 (15%)
Shoulder	18 (5.5%)
Elbow	6 (1.9%)
Hand	18 (5.6%)
Lower limb	58 (18%)
Hip	18 (5.6%)
Knee	23 (7.3%)
Ankle	26 (8.1%)
Foot	15 (4.6%)
Spine	18 (5.6%)

At least one ADR was reported in 7,9% of players: 3,5% reacted to NSAIDs, 2,6% to antibiotics, 1% to analgesics and one case was reported as a reaction to supplements (**table IV**). For intra-articular injections, only 2 players (0.5%) experienced a knee effusion and a skin rash.

Table III *- Permitted drug use in soccer players in last years and frequency.*

ADRs	n	(%)
Non-steroidal anti inflammatory	13	3.5
Analgesics	4	1
Antibiotics	10	2.6
Supplements (minerals, supplements, vitamins)	1	0.3
Intraarticular (CCS, hyaluronic acids, platelets gel)	2	0.5

Table IV *- ADRs to permitted drug in soccer players.*

Drug category	Users n (%)	Current (> 30 d/y) users n (%)
Non-Steroidal Anti Inflammatory (NSAIDs)	344 (91%)	116 (33.7%)
Analgesics	242 (64%)	75 (31.8 %)
Antibiotics	197 (52,1%)	11 (5.6 %)
Supplements (minerals, supplements, vitamins)	374 (98.9%)	302 (80.7 % daily)
Topic-intraarticular Drug	Users n (%)	Current (> 30 d/y) users n (%)
Platelet gel	34 (8.9 %)	15 (44.1%)
Hyaluronic acid	84 (22.3 %)	39 (46.4%)
Steroids	8 (2 %)	3 (37.5 %)
Others	11 (2.8 %)	4 (36.3%)

The largest number of ADRs may be predictable as non immunologic reactions while a non-negligible percentage is immunologically mediated drug hypersensitivity. In 30% of the players, reactions as urticaria-angioedema syndrome or more severe conditions as bronchospasm or anaphylaxis (1 case reported) have been found (**table V**).

Discussion

Literature interest in relationship between physical activity, chronic inflammatory diseases and drug use is increasing. Particularly, we have previously showed how a regular and intense physical exercise could prevent or reduce the risk of developing a chronic inflammatory skin disease such as psoriasis in both non agonist as well as agonistic (Italian second soccer league) athletes (15, 16). However, information regarding drug use/abuse and related ADR are still limited. The data obtained in our survey show a significant use (< 90%) of permitted drugs among professional second league soccer players. The most frequently used drugs were NSAIDs: approximately, one third of the players had NSAIDs almost 1 time per month during the previous year. Antibiotics and analgesics were used less frequently. A regular use of supplements was observed in a great number of players (80%): the identification of the chemical composition of the supplements was regretfully impossible due to the high number of products consumed.
In agreement with a previous similar cross-sectional study, our data confirmed an ongoing tendency to abuse drugs (8), in our cohort of players independently from doctor's advice. In fact in most cases, the use and abuse of permitted drugs is not justified by acute or chronic diseases or events, or by appropriate medical prescription. It is also widely known that soccer determines an increasing number of traumas and injuries (17), especially when practiced at a competitive level. The highest rate of these events is usually restricted to the sport season, throughout fall, winter, and spring (18). Interestingly, our data indicate that 80% of players reported drugs intake during their whole career, but less than a half

Table V *- Type of ARDs to permitted drug in soccer players.*

Type of ADRs n (%)	Non Immunomediated	OAS	Bronchospasm	Anaphylaxis
Non-steroidal anti inflammatory	7 (54%)	3 (23%)	2 (15.5%)	1 (7.5%)
Analgesics	3 (75%)	1 (25%)	0 (0%)	0 (0%)
Antibiotics	8 (80%)	2 (20%)	0 (0%)	0 (0%)
Supplements (minerals, supplements, vitamins)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Intraarticular (CCS, hyaluronic acids, platelets gel)	1 (0.3%)	1 (0.3%)	0 (0%)	0 (0%)

of them had suffered injuries during the previous year. Clearly, such a wide use of NSAIDs and analgesics among elite soccer players is justified by real necessity but likely becomes a habit of self-administration, especially in terms of prolonged duration of treatment, combo therapy and/or prophylactic use devoid of any medical rationale. Actually, self drug administration appears to be common practice among athletes and often drugs are taken spontaneously, without seeking medical advice (7). This happens for extended periods (19) of time and beyond the recommended doses, furthermore in associations with many other drugs without given therapeutic evidences. Our data clearly show that NSAIDs and painkiller as well as of supplements are the classes of drugs that fit into the picture of self-administration. Obviously, this attitude exposes the players to an increased ADRs risk, especially following incorrect drugs intake and combination. Increased risk of ADRs is not predictable reactions – *i.e.,* those related to the substance pharmacologic action –, but is also unfortunately unpredictable because reactions depend on genetic factors, patients' immunological response, *etc*.

As a matter of fact, many ADRs (30%) are unpredictable and immune-mediated. It has been reported an increased incidence ADRs following an inappropriate use of NSAIDs (13). Moreover, this kind of reaction not only exposes to clinical manifestations or organ-specific symptoms such as urticaria-angioedema (23%), prurigo, rhinitis, but also to life threatening systemic manifestations such as bronchial spasm (6.6%), hypotension and anaphylaxis (in our cohort observed in 1 case).

As observed for systemic drugs our analysis highlights use and abuse of intra-articular substances as well. Intraarticular injections are commonly used in practice to treat chronic pain due to traumatic or degenerative diseases. Use of intraarticular drugs among soccer players has not been fully investigated and only few cases are reported (20, 21). In our cohort one third of soccer players (29.2%) were treated at least once with intraarticular injections and 9.3% used intraarticular drugs in the previous year. Although few ADRs were attributed to intraarticular treatment, 7-9% of patients experienced, especially during the first and second cycle, local ADRs like flares, effusions (22), skin rashes (23) and skin necrosis (24). Local reactions occurred significantly more often in patients who received more than one course of treatment than in patients who had received only a single course of treatment (25). Moreover, serious systemic adverse events like septic arthritis, disorders of the nervous system, of the circulatory (26) and musculoskeletal system (27) have been described. Episodes of anaphylactic shock were also reported (28). In our study, we observed only 2 cases (0.6% of all cohort) of mild ADRs related to intraarticular drug use.

In the last 30 years, doping has been largely described and has taken a center stage in the public attention. This has raised a significant awareness of the problem and has led to the development of detection methods for regular monitoring in sports. Nevertheless, this drug misuse/abuse still happens in professional and amateur sports not only involving medications considered as performance-enhancing. Our survey shows a better knowledge and raised awareness about the problem of sport medicalization, now perceived as a problem but only for its impact on performance and sport activity. The awareness about doping harming athletes' health is still not fully developed. This finding is enforced by data showing that drug use/abuse has not changed much in the last decade (8).

An effort by sport institutions is warranted in raising awareness in the sport community overuse/abuse of drugs and their impact on the athletic performance and on the risks and implications for the athlete's health. The education should start likely at school level, in pediatric and young adult age, when the recreational aim of sport should go at the same pace with the educational purpose.

Conclusions

This study shows that drug misuse/abuse in professional soccer players is an emerging issue, especially with regards to NSAIDs, exposing athletes to predictable or/and unpredictable risks for their health. In the future, it is of crucial importance to foster the athlete's awareness of these problems through a continuous education, starting from the very beginning of sport practice.

Conflict of interests

The authors declare that they have no conflict of interests.

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Diagnostic dilemmas of Titanium Hypersensitivity in patients with medical implants: a case series

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Key words

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To the editor,

Titanium is used in numerous medical, dental devices and implants. It is considered a "hypoallergenic" alternative when there is concern for hypersensitivity to other metals (1-4). Nevertheless, titanium hypersensitivity (TH) reactions have been reported in association with cardiac pacemakers and dental, cardiac, orthopedic, cardiovascular and neurosurgical implants (4-12). Metal allergy is traditionally considered a type IV hypersensitivity reaction. Type I hypersensitivity to metals is rare, but such reactions have been reported, including to titanium (13-16).
This case series highlights the diagnostic dilemmas associated with TH and emphasizes that a high index of suspicion for TH is required for prompt diagnosis and management of postoperative complications associated with TH reactions.

A retrospective review of four patient records with postoperative reactions following insertion of a titanium-containing implant was performed. The study complied with the ethical standards established in the Declaration of Helsinki of 1946.

Case 1

A 53-year-old African American female was referred for patch testing for suspected metal allergy to lumbar metal implants placed for degenerative disc disease. Three months after her procedure, she experienced worsening lower back pain, bilateral leg pain and paresthesias, as well as erythema, warmth and tenderness overlying the midline surgical scar. Lumbar computed tomography (CT) myelogram and magnetic resonance imaging (MRI) were unremarkable. Laboratory results including complete blood count,

tissue cultures and erythrocyte sedimentation rate were also within normal limits. Synthes titanium alloy (TAN) rods and screws (titanium, 6% aluminum, 7% niobium and 0.02% nickel) had been used for the spinal fusion. Patch testing with the standard North American Contact Dermatitis Group (NACDG) standard series and Chemotechnique extended metal series was not tolerated due to an asthmatic reaction with facial angioedema within 24 hours of patch placement, requiring an emergency room visit for treatment with systemic corticosteroids. Limited patch tests to nickel and titanium were subsequently performed. Titanium nitride 5% (Chemotechnique extended metal series) was placed on the patient's right forearm and nickel sulfate 2.5% (NACDG standard series) was placed on the left forearm. After 72 hours, the patient demonstrated a 1+ reaction to nickel and no reaction to titanium; however, the patient reported a symptomatic reaction (pruritus) at the titanium patch test site prior to her visit. Thus, prick testing was also performed to assess immediate hypersensitivity to nickel and titanium. The skin on the right upper arm was pierced in three spots, patches were applied (nickel 2.5%, titanium 10%, and petrolatum, a control) and the patient was monitored for one hour. Urticarial reactions were observed at both the nickel and titanium sites, but not at the petrolatum control site. Prick testing of a healthy control to the same metals (nickel 2.5%, titanium 10%) was negative. The patient later disclosed a history of suspected metal allergy after a failed left foot bunionectomy required removal of titanium screws used during the procedure, although testing to evaluate for metal allergy had not been performed.
After confirming the presence of a metal allergy, the spinal implant was removed and replaced with bone morphogenic protein. Prior to wound closure, gram stain, cultures, and tissue samples from the wound and hardware were obtained and were negative. Pathology of the tissue samples revealed fragments of necrotic bone with inter-trabecular fibrosis and chronic lymphocytic inflammation, consistent with aseptic lymphocyte dominated vasculitis associated lesion (ALVAL, not shown). Following implant removal, the patient experienced resolution of her symptoms.

Case 2

A 42-year-old Caucasian female presented for evaluation for metal hypersensitivity reactions to cervical metal implants due to persistent neck pain and erythema of the anterior neck with delayed wound healing along the posterior incision site one year after the procedure. There was a history of multiple cervical spinal fusions/reconstructions due to pseuduoarthrosis and hardware failure. CT of the cervical spine demonstrated lucency around the hardware, suggestive of hardware failure. Globus Coalition titanium alloy (TAV) implants (titanium, 6% aluminum, and 4% vanadium) had been used for the anterior cervical discectomy and fusion. Synthes titanium alloy (TAN) rods and screws (titanium, 6% aluminum, 7% niobium and 0.02% nickel) were used for the posterior spinal fusion and a Synthes SynMesh vertebral body replacement device was inserted anteriorly. Patch testing was performed using solid metal samples (*i.e.* discs) of the Synthes plate and rod and the Globus plate. The discs were placed on the skin after cleaning with an alcohol pad and covered with cloth tape. At 24 and 48 hours, no reactions were observed. At 72 hours, few erythematous macules were observed under the Synthes and Globus plate samples. At one week, small, grouped, pruritic erythematous papules were observed under the Globus plate sample. No reaction was observed with the titanium rod sample. Patch testing with these plates on a healthy control did not result in any reactions. The allergy testing suggested a hypersensitivity to the Globus implant and the location of these implants appeared to be consistent with the anterior neck erythema the patient had developed postoperatively. The patient underwent removal of the Globus implants, which were replaced by structural allograft spacers, and recovered without complication.

Case 3

A 57-year-old Caucasian female was referred for evaluation for metal hypersensitivity to a cervical metal plate, which had been placed for degenerative disc disease. She developed erythema and edema of the face, neck, and upper chest shortly after the procedure, along with shortness of breath from a pleural effusion that began at the same time. Her symptoms did not resolve with antibiotics or systemic corticosteroids and she subsequently required multiple thoracenteses, which repeatedly revealed a highly eosinophilic transudate. The cervical plate used was a standard alloy composed of titanium, 6% aluminum and 4% vanadium (Ti6Al4V, TC4). Patch testing to NACDG standard series and Chemotechnique metal series showed only a positive 1 + reaction to gold sodium thiosulfate. Prick testing to an arbitrarily selected titanium salt (titanium IV oxide 10%/Chemotechnique) revealed an urticarial reaction within one hour. Repeat prick testing was performed using six titanium preparations (titanium IV oxide 0.1%, titanium 1%, titanium III nitride 5%, titanium III oxalate decahydrate 5%, titanium 10%, titanium dioxide 10%) as well as aluminum and vanadium. This revealed urticarial reactions to five of the six titanium salts (no reaction to titanium dioxide 10%). Prick testing with these titanium salts in healthy controls (N = 20) was negative. The patient was diagnosed with type I hypersensitivity to titanium and subsequently underwent removal of the plate in her neck with resolution of her symptoms within two months. The patient remains symptom free more than four years later.

Case 4

A 61-year-old Caucasian male presented for evaluation of a pruritic rash on his right knee (**figure 1 a**). The patient had developed the rash shortly after undergoing a right total knee replacement six months prior to presentation. He had been experiencing per-

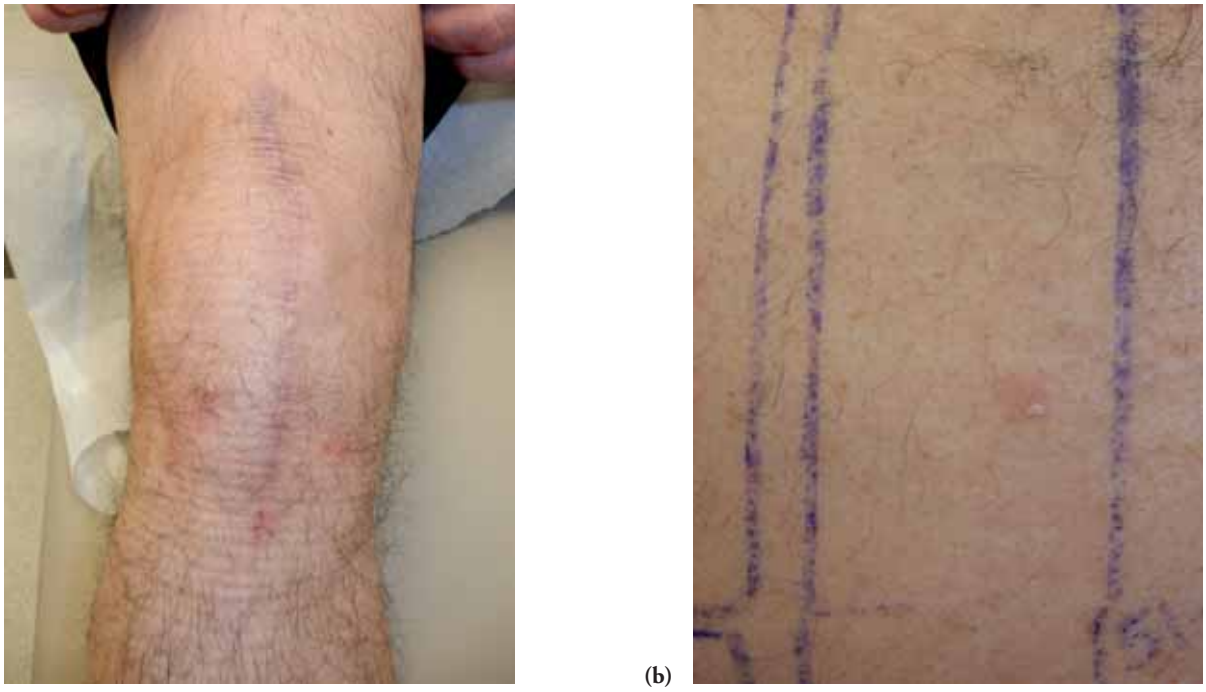
sistent right knee pain since the surgery, longer than the expected period of postoperative pain. A Triathlon Titanium implant (cementless) was used, a highly porous metal biologic fixation technology created using 3D-printing technology during which multiple layers of titanium powder are melted together. Physical exam demonstrated an ill-defined erythematous plaque localized around a well-healed linear scar on the right knee (**figure 1 a**). Patch testing to the NACDG standard series, Chemotechnique extended metal series, and dental series showed a positive +/- reaction to titanium III oxalate decahydrate at 48 hours and 1+ reaction at 72 hours (**figure 1 b**). The patient was diagnosed with TH secondary to recent implantation and was given triamcinolone 0.1% cream for symptomatic relief. The patient was counseled on the diagnosis and replacement of the implant was recommended. He declined revision of the knee replacement to avoid an additional surgical procedure. He has continued to experience chronic dermatitis with recurrent flares, controlled with topical corticosteroids, but has persistent knee pain.

We report four cases of suspected TH, including two cases of type I hypersensitivity reactions. The clinical manifestations consistent with metal hypersensitivity, positive allergy testing, absence of a more likely diagnosis, and dramatic resolution of symptoms with explanation (in three of the four cases) support the diagnosis of TH. Additionally, for Case 1, there was peri-implant tissue for histologic study at the time of implant revision, which revealed the presence of ALVAL, one of the histologic biomarkers of metal hypersensitivity reactions (17, 18).

The prevalence of TH is difficult to assess due to lack of reliable detection methods; however, it is estimated to be 0.2 to 1% (16, 19, 20). In a study by Sicilia *et al.* (16), 35 patients at a dental clinic with symptoms consistent with TH and underwent patch testing and prick testing. The majority of patients with positive allergy testing had developed both type I and type IV hypersensitivity (positive patch and/or prick test) reactions to their titanium implants (16). The results of this study, in addition to the present cases, suggest TH reactions may be different compared to that of other metals.

While patch testing is considered the standard method for detecting metal allergy, its use for TH is not as well accepted (1-3). Positive patch test reactions to titanium are rare and a negative patch test may not exclude a diagnosis of TH. Current patch test formulations of titanium salts may not be adequate for the detection of type IV hypersensitivity to titanium because of low epidermal penetration (19, 21). Patients with a negative reaction to titanium on patch testing may still be diagnosed with TH based on positive reactions on other diagnostic tests and/or resolution of postoperative symptoms after removal of the titanium-containing implant (1, 3-5, 7, 9, 12, 14, 15) Case 4 was the only patient who

Figure 1 - (**a**) *Subacute dermatitis on the knee, adjacent to and overlying surgical scar seen in Case 4.* (**b**) *Patch test reaction to titanium (III) oxalate decahydrate at 72 h, revealing a 1 + reaction.*



(a) **(b)**

demonstrated a positive reaction to titanium with standard patch testing, allowing for the unambiguous diagnosis of TH. Case 2 was diagnosed via patch testing, although samples of the metal implant itself (discs) were used. The positive skin test to the disc and the resolution of signs and symptoms after removal suggest that the titanium allergy was relevant to the patient's favorable outcome. Testing with metal discs has been discouraged because of concerns about false negative patch testing (22). Despite these concerns, this patient exhibited a type IV hypersensitivity reaction to the disc, which was the basis for implant removal.

Thyssen *et al*. (23) proposed ten objective criteria that support implant-related metal hypersensitivity, four of which are considered major criteria: (1) eruption over the implant; (2) positive patch test to metal; (3) chronic dermatitis; (4) resolution of symptoms after removal of the implant. Three of the cases presented in this series did not fulfill all of the major criteria needed for the diagnosis of implant-related metal hypersensitivity, but, underwent implant removal with resolution of symptoms, supporting the diagnosis of TH. We suspect the patient in Case 4 would have also experienced improvement and/or resolution of symptoms with explantation, but the patient refused an additional procedure. Therefore, while these criteria are helpful in diagnosing implant-related metal hypersensitivity in general, they may or may not apply to TH and may be less sensitive for type I hypersensitivity.

This series adds to the literature on metal hypersensitivity reactions to titanium, suggesting that patch testing alone may be insufficient to confirm TH, and that prick testing should be considered to confirm the diagnosis of TH. We propose prick testing or alternative type I hypersensitivity testing methods for patients who present with type I hypersensitivity-like reactions or when patch testing is negative. As titanium presents unique challenges in patch testing, these additional testing methods may increase the sensitivity of allergy testing and allow for more reliable diagnosis.

Conflict of interests

The authors declare that they have no conflict of interests.

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Chronic spontaneous urticaria treated with omalizumab: what differentiates early from late responders?

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Key words

Chronic Urticaria; omalizumab; biomarkers; IgE; D-dimer; therapy.

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To the editor,

Omalizumab is the first choice treatment for severe Chronic Spontaneous Urticaria (CSU) patients who are unresponsive to second-generation antihistamines even at higher than licensed dose (1). The drug is effective in about 85% of patients, inducing a dramatic drop in UAS-7 levels. The clinical effect can be very rapid (in about 70% of patients, the so-called early or fast responders, the effect may appear as early as 3-5 days after the first administration), or slow (in about 15% of patients, the so-called late or slow responders, 3-4 months of treatment are needed to see a benefit). The drug is ineffective in the remaining 15% (2). Several recent studies have shown the association between elevated baseline total IgE levels and a positive response to omalizumab, with non-responders showing significantly lower IgE (3-5). A possible "auto-allergic" pathogenesis mediated by IgE specific for self-proteins may reasonably explain a rapid response to omalizumab in some patients with CSU (6, 7). In contrast, the reasons why a proportion of patients take months to respond to the drug are less clear. This subpopulation might coincide with patients showing an IgG-mediated autoimmune process able to activate mast cells and basophils via the high affinity IgE receptor, either directly (by IgG-anti-FceRI) or indirectly (by IgG directed against receptor-bound IgE) (8, 9). In this case, the effect of omalizumab would rely on the down-regulation of IgE receptors, a process that would take some months (10). From the clinical point of view, patients showing a rapid or slow response to omalizumab have not been compared so far. The present study investigated the clinical and serological features in these two subsets of CSU patients.

One hundred and thirty patients (M/F: 53/77; mean age: 50,6 years (range 13-89 years)) with severe CSU (UAS-7 > 30) unresponsive to second generation antihistamines at any dosage and successfully submitted to treatment with omalizumab 300 mg/month for at least 3 months were studied. Based on their response to Omalizumab, patients were classified as early responders (drop of at least 50% of UAS-7 from baseline level already 1 month after the first administration; n = 108) and late responders (no appreciable clinical effect one month after the first administration, but drop of at least 50% of baseline UAS-7 after 3 months of treatment; n = 22). Age, sex, disease duration, and several baseline clinical parameters including ESR, CRP, thyroid autoimmunity, total IgE , D-dimer, and atopic status (as assessed by SPT with a large panel of commercial extracts of both seasonal and perennial respiratory allergens) were compared between

the two subsets. The Chi-Square test with Yates' correction, the two-tailed Student's t-test, or the Mann-Whitney non-parametric test were used where appropriate. Probability values less than 5% were regarded as statistically significant. The internal review board approved the study, and all the patients signed an informed consent to use their clinical data in an anonymous form. The study subpopulations are compared in **table I**. The two groups did not differ in gender, mean age, disease duration, atopic status, inflammation markers, and thyroid autoimmunity. In contrast, early responders showed a significantly higher proportion of patients showing elevated (> 100 UI/ml) baseline total IgE (67% *vs* 33%, respectively; $p < 0.05$). Of those showing elevated total IgE at baseline, only 16/41 (39%) and 2/5 (40%) were atopic among early and late responders, respectively. Although median total IgE levels were much higher among early responders (181 UI/ml *vs* 45 IU/ml for early and late responders, respectively), probably due to the small number of late responders the difference between the two subgroups did not reach the statistical significance.

To the best of our knowledge, this is the first study comparing the clinical features of the two specific subsets of CSU patients responding to anti-IgE therapy, namely the early and late responders. The two subsets of omalizumab responders were virtually identical with the only difference of a much larger proportion of patients showing elevated IgE levels in the early responders group. This observation is in keeping with previous studies showing the direct relationship between elevated IgE levels and the response to anti-IgE treatment in the general CSU population (3-5). It is conceivable that in these patients elevated total IgE may mirror the predisposition to synthesize autoreactive IgE antibodies that the anti-IgE mAb rapidly binds and eliminates from the circulation. It has also been shown that omalizumab is able to bind IgE fixed to the high affinity receptor and to detach them (11), which leads (in a long term) to a down-regulation of the receptor. In patients showing a slow response to omalizumab it is possible that this latter mechanism of action is the most important. In effect, a very recent study seems to suggest that the contemporary presence of IgE and IgG-mediated autoimmunity (particularly against the high affinity IgE receptor) may slow down the clinical response to the drug (12).

Table I *- Clinical and serological characteristics of patients showing a prompt or late response to omalizumab.*

	Early responders (n = 108)	**Late responders (n = 22)**	**p**
M/F	44/64	9/13	NS
Mean age (years)	49,7	54,8	NS
Median disease duration in months (range)	12 (2-600)	10,5 (2-300)	NS
Atopic status (%)	30 (27%)	9/22 (41%)	NS
Thyroid autoimmunity (%)	20/98 (20%)	3/21 (14%)	NS
D-dimer > 500 ng/ml	42 (39%)	9/21 (43%)	NS
Total IgE > 100 IU/ml	41/61 (67%)	5/15 (33%)	< 0.05
Median IgE level	181 (9-1139)	45 (5-1000)	NS
Elevated CRP or ESR	24 (22%)	4 (18%)	NS

Conflict of interests

The author declares that he has no conflict of interests.

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