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Patient Reported Outcomes in allergic diseases: findings and implications

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Access to the patient’s perspective gives a unique key to enhances the range of outcomes that can be evaluated beyond the traditional clinical, biological and functional measures. Patient Reported Outcomes (PROs)—any report directly provided by patients on their experience with health and treatments—are now recognised as an essential part of clinical management and research studies (1). They assess a range of outcomes such as Health Related Quality of Life (HRQoL), level of control, satisfaction, awareness, disease perception. The role of PROs gets more significance when interventions have similar effects in terms of efficacy and safety, and is crucial in any management paradigm that relies on personalized medicine. Moreover, regulatory agencies encourage the inclusion of PROs in the process for approval of new drugs and treatments (1).

Over the last two decades, PROs have emerged as a critical tool for assessing disease impact, level of control and response to treatment in allergic patients. Specifically, the integration of PROs in the healthcare continuum from research to clinical practice, has supplied deep insights into care and management of allergic diseases.

With great interest we read the articles of Dortas Junior (2), El-Qutob (3), and Leiria-Pinto (4), published in this issue of European Annals of Allergy and Clinical Immunology. Each of them develops one of the three steps of the FDA Roadmap to Patient-Focus Outcome Measurement (5) (figure 1).

The observational study by Dortas Junior et al. (2) is the first-time evaluation of spirituality in patients with chronic urticaria (CU), contributing to better understand the disease (figure 1, step 1). During the last decade, the role of this PRO in chronic diseases has received more attention, with growing evidence that this dimension of subjective experience is related to physical and psychological wellbeing and contributes to the health outcomes (6). In a study sample of 100 patients, Dortas Junior and co-workers, by mean of validate questionnaires, evaluated the spiritual wellbeing in CU patients with different levels of disease control, and the relationship between spiritual wellbeing and HRQoL. In their analysis the authors highlight how subjects with poorly controlled CU had significantly lower spiritual wellbeing and worse HRQoL than subjects with controlled CU. Moreover, higher levels of spiritual wellbeing were associated to better HRQoL scores. This study suggests that spirituality may play a role in the experience of CU patients. Additional research is needed to better investigate this construct, and therefore planning targeted intervention programs designed to manage the spiritual well-being of patients with CU.

El-Qutob and co-workers (3) provide the results of the first study that explores the effectiveness of specific immunotherapy based on the combination of allergens in polysensitized patients with asthma and/or rhinitis (figure 1, step 2). Disease control and HRQoL were retrospectively evaluated by mean of validated tools in 39 patients observed in clinical practice. The authors found that the administration of the combination vaccine significantly increases the level of asthma control and HRQoL, and maintains the improvement at 12 months. Although preliminary, these findings are important because highlight for the first time how patients experience specific benefits and changes during the first year of treatment with immunotherapy based on the combination of allergens.

The availability of simple, psychometrically sound questionnaires should help to implement the assessment of PROs in daily practice. To be used in languages different from the original one, PROs measures must be cross-culturally adapted and their psychometric characteristics must be verified, following well-established procedures. The Test for Respiratory and Asthma Control in Kids (TRACK) is the only available tool for the assessment of respiratory control in children younger than five years. It was originally developed and validated in English, and then cross-culturally validated into other languages. The article
of Pinto et al. (4) describes the results of the rigorous procedure they followed to adapt and validate the TRACK into the Portuguese language (figure 1, step 3). First, the proper forward-backward translation methodology was adopted to obtain a conceptual and linguistic version that is equivalent to the original one. Second, internal consistency, criterion validity, construct validity, test-retest reliability, and responsiveness were determined. The results of the validation analyses show that the Portuguese version of TRACK has satisfactory psychometric properties for the assessment of respiratory control in pre-school children. Taken together, these studies offer innovative contributions, all of which provide insights into how researches can capture the disease experience of allergic patients and ensure a comprehensive assessment of disease and drugs impact.

References


The diverse roles of T cell subsets in asthma

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Summary

T cells are coordinators of the immune response and have been shown to play a central role in the pathophysiology of asthma. A good understanding of the T cells functions in asthma is important for therapeutic reasons, in particular for the choice of biological treatments in severe asthma. Although classically considered a Th2 disease, it is now clear that other types of T cells contribute for the pathophysiology and the heterogeneity of asthma. We here review how the different subsets of T cells are involved in the different phenotypes/endotypes of asthma and how this may influence the treatment of the disease.

Impact statement

This review highlights the heterogeneity of asthma and the significant role of other types of T-cells aside from Th2 (including unconventional subsets) in its development and symptoms.

Introduction

Asthma is a heterogeneous disease. Several distinct clinical phenotypes have been described and it is now debated whether asthma is a “single disease” or, instead, a group of inflammatory lung diseases with similar manifestations but diverse pathophysiology. More recently, these underlying inflammation mechanisms have been used to classify asthma into “endotypes” (1). Although several markers have been proposed to distinguish specific asthma endotypes (e.g., IgE sensitization in allergic asthma, peripheral eosinophil counts in eosinophilic asthma, periostin in Th2-high asthma, etc.), the most consensual distinction is based on the CD4+ T-helper (Th) cytokine profiles involved (i.e., Th2-high vs Th2-low), therefore illustrating the central role of these cells in asthma.

T cells are central coordinators of the immune response. T-cell receptor (TCR) recognition is crucial for the initiation and specificity of the adaptive immune response, while T-cell derived cytokines tailor the type of immune response. Asthma is an inflammatory lung disease and T cells have been shown to play a central role.
role in the pathophysiology of the disease. A good understanding of T cells in asthma is also important for therapeutic reasons, in particular for the choice of biological treatments in severe asthma. T cells are generally divided into two major types: CD4+ T cells and CD8+ T cells. CD4+ T cells are activated by antigen presented by major histocompatibility complex (MHC) class II on the surface of professional antigen-presenting cells (APC), whereas CD8+ T cells recognize antigens presented by MHC class I on the surface of all nucleated cells. As discussed below, each of these types can be subdivided into several functionally distinct subtypes.

Classically, asthma has been considered a Th2-mediated inflammatory disease. However, more recent studies have identified contributing roles for other types of T cells in the pathophysiology and the heterogeneity of the disease. In this chapter, we review how the different types and subtypes of T cells are involved in asthma and its phenotypes/endotypes.

Types of T cells and their roles in asthma

αβ CD4+ T cells

Also termed “helper T cells” (or Th cells), αβ CD4+ T cells have the capacity to produce high quantities of cytokines and influence a variety of immune cells to determine effector immune responses. Naïve Th cells (Th0) are produced in the thymus without a defined cytokine profile. Matured dendritic cells (DCs) present antigens in MHC class II to naïve Th0 cells that bear TCRs specific for the antigen and, together with co-stimulation (e.g., CD80, CD86, OX40L) and cytokines, stimulate T cell proliferation and differentiation into effector Th polarizations (defined by the major profile of cytokine production). From then on, Th cells may behave to promote inflammation (e.g., Th1, Th2, Th17, etc.) or regulation (e.g., Tr1, Foxp3+ induced-Tregs).

It is now over 30 years since the original Th1/Th2 functional distinction of CD4+ T cell subsets was published by Mosmann (2, 3). Th1 cells are polarized by IL-12, express the transcription factors t-bet and STAT4, and produce high amounts of IFN-γ and IL-2 to stimulate macrophages, CD8+ T cells, IgG B cells, and IFN-γ CD4+ T cells to combat intracellular bacteria and protozoa. On the other hand, Th2 cells are induced, upon activation, by the presence of IL-4 and IL-2, and the key transcription factors for the Th2 program are STAT6 and GATA3. Th2 cells produce high amounts of IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF (encoded in the IL-4 gene cluster on chromosome 5q31) and stimulate eosinophils, basophils, and mast cells as well as B cells, and the humoral adaptive response (antibody production), typically against extracellular pathogens.

Th2 cells

Ever since Mosmann (2), the functional Th1/Th2 divide has been used to classify inflammatory diseases according to the predominant type of cells and/or cytokines. The majority of asthma patients are atopic and have an allergic pattern of inflammation in their airways. Th2 cells were identified as the primary cells involved in allergic asthma more than 25 years ago (4). Mice deficient in t-bet (the Th1 master-regulator transcription factor) develop spontaneous allergic/Th2 eosinophilic airway inflammation and hyperreactivity (5), while knocking-down GATA-3 (the Th2 transcription factor) prevented airway hyperreactivity and inflammation (6). Many other compelling data in humans and mice have demonstrated the central role of Th2 cells in allergic asthma (7, 8). Th2 cytokines are main drivers of allergic inflammation in atopic asthma (8) and have been associated with the activity of disease, symptom scores, airway eosinophilia, and bronchial hyperreactiveness (7). IL-4 upregulates the Th2 transcription factor GATA-3 in naïve T helper cells and is therefore essential for Th0 to Th2 differentiation and the production of IL-5, IL-9, IL-13, CCL17 and eotaxin (a pivotal chemokine in eosinophil recruitment). IL-4 also increases B cell proliferation and differentiation to plasma cells, and it increases antibody production and isotype class-switch to IgE. IL-5 also helps B cells differentiation and isotype class switch to IgG acts, promotes basophil differentiation and histamine release, and it is a crucial cytokine in eosinophils function (promoting the production, differentiation, recruitment, activation and survival of these cells). IL-13 shares some functions with IL-4, but it also stimulates goblet cell hyperplasia and mucus production, and increases smooth muscle contractility and bronchial hyperreactivity. It is noteworthy that Th2 cells are involved also in some non-atopic asthma patients (9). Many patients with high eosinophil count in sputum and blood do not show increased total IgE or allergen-specific IgE. This subset of asthma has been termed “eosinophilic” asthma and is also believed to be Th2-driven (10).

Several monoclonal antibodies targeting Th2 cytokines have shown great benefit in severe asthma, both allergic and/or “eosinophilic”, once more demonstrating the importance of Th2 cells and their cytokines in the physiopathology of these asthma endotypes. Currently, there are 5 monoclonal antibodies approved by the European Medicines Agency of the European Union, (EMA) and the Food and Drug Administration of the United States of America (FDA) for the human treatment of Th2-mediated asthma:

1. Omalizumab targets the Cε3 domain of free IgE and prevents IgE interaction with the high-affinity receptor (FcεRI) on mast cells, basophils, eosinophils, Langerhans cells and dendritic cells. Although not directly targeting Th2 cells or cytokines, it is well recognized that IgE production is a result of Th2 activation and IgE-mediated degranulation and activation of mast cells and basophils is responsible for late phase Th2-type inflammation. Omalizumab was approved for the treatment of moderate-to-severe allergic asthma by the FDA in 2003 and the EMA in 2005;
2. Mepolizumab and Reslizumab directly bind to and neutralize the cytokine IL-5, limiting its availability for eosinophils and other cells. Mepolizumab received approvals for severe refractory eosinophilic asthma by the FDA and EMA in 2015, while reslizumab was approved for similar indications by the FDA and EMA in 2016;

3. Benralizumab binds to the α-subunit of the IL-5 receptor (IL-5Rα) targeting the cells that express it for destruction by antibody dependent cell cytotoxicity. Benralizumab received approvals by FDA in 2017 and EMA in 2018 for severe eosinophilic asthma;

4. Dupilumab targets IL-4Rα, therefore inhibiting both IL-4 and IL-13 signaling. It was FDA and EMA approved in 2019 for asthma with type 2 inflammation (characterized by raised blood eosinophils and/or raised FeNO).

It should be borne in mind Th2 cytokines are produced by conventional αβ CD4+ T cells but also other T cells, such as “unconventional” T cells (i.e., iNKT or γδ T cells, as discussed below), and even from other non-T immune cells.

The interplay between the bronchial tissue and the adaptive immune system in asthma has been the focus of recent interest. Several cytokines produced by the tissue may play a role in the initiation and amplification of Th2 inflammation in asthma, among them thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and osteopontin (11, 12). TSLP is produced by epithelial cells in response to proinflammatory stimuli (such as allergens, pollutants, infections, etc.) and acts on dendritic cells to upregulate surface OX40 ligand to promote Th2 differentiation (13). TSLP is increased in the airways of patients with asthma and correlates with the severity of disease. IL-25 is produced by tissue eosinophils and basophils and synergizes with TSLP to increase GATA-3 expression in memory Th2 cells stimulating cell proliferation and cytokine production (14). IL-25 may also act on innate cells to stimulate the production of Th2 cytokines independently of T cells (e.g., nuocytes (15)). Also, IL-33 is produced by airway epithelial cells, fibroblasts and smooth muscle and induces dendritic cells production of IL-5 and IL-13, therefore stimulating Th2 polarization. IL-33 expression is also increased in the airways of patients with asthma (16).

The clinical importance of these tissue-derived Th2-stimulating cytokines in asthma has also been explored using blocking monoclonal antibodies in vivo. Two phase II trials with the monoclonal antibody tezepelumab/AMG 157, a human IgG2λ mAB targeting TSLP, showed promising results in severe asthma: reducing the levels of blood and sputum eosinophils, FeNO and allergen-induced bronchoconstriction (17), and reducing the annualized asthma exacerbation rates by 61-71%, regardless of blood eosinophil levels at enrolment (18). Two phase III trials with tezepelumab are currently ongoing.

**Th1 and Th17 cells**

Importantly, not all patients with asthma present high levels of Th2 cytokines, local or systemic eosinophilia, or allergic sensitization. These patients usually present a predominance of neutrophils over eosinophils in the airway; therefore, this subset of asthma has been designated “neutrophilic” or “intrinsic”, and more recently “non-Th2” or Th2-low”. In “non-Th2” asthmatics, disease symptoms tend to initiate later in life, present a more severe clinical evolution, worse response to corticosteroids, and less reversible airway obstruction. It is believed to be mediated by Th1 and Th17 cells (19). IFN-γ-producing Th1 cells have been found among the bronchoalveolar lavage (BAL) T cells in asthma patients (20). Also, Th2-independent triggers, such as viruses, air pollution, and exercise, can induce and/or exacerbate asthma symptoms in some patients, and the serum levels of IFN-γ increase during acute asthma exacerbations. Th1 may be involved also in children with severe asthma, in which memory CCR5+ Th1 cells were shown to be the most significant type of CD4+ T cell in the bronchoalveolar lavage (BAL), together with Th17 cells and IFN-γ + IL-17 + double positive cells, independently of the allergic status (21). The therapeutic effect of IFN-γ blockade in asthma has not been assessed in humans so far.

Th17 cells are induced by a combination of TGF-β and IL-6, leading to the expression of the transcription factor RORγT, and are characterized by the production of IL-17A, IL-17F and IL-22. These cytokines can induce epithelial cells, endothelial cells, fibroblasts, neutrophils and eosinophils to produce IL-6, GM-CSF, CXCL10 and CXCL8, therefore stimulating “neutrophilic inflammation”. Th17 cells are known to regulate neutrophilic and macrophage inflammation in autoimmune diseases and, more recently, have been implicated in neutrophilic airway inflammation (22), in asthma, and in corticosteroid insensitivity (23). Others have found that IL-17A and IL-17F in asthma did not correlate with neutrophilic inflammation (24). Circulating Th17 cell numbers and IL-17 plasma concentrations are increased in asthmatic patients and correlate with disease severity. In mouse models of severe asthma, specific roles for Th1 cytokines (IFN-γ gene knock-out) were demonstrated in airway hyperresponsiveness (AHR) but not in airway inflammation while IL-17RA knock-out mice developed AHR but airway inflammation was lower (25). In allergic asthma, the role of Th17 cells has not been fully elucidated and contradictory results were found in mouse models. IL-17 may play a dual role: it seems to be essential during allergen sensitization (26), but later, once sensitization is established, IL-17 attenuates the allergic response and may reduce asthma chronic manifestations (26). More recently, it was described in humans a novel subset of CD4+ T cells with memory and effector phenotypes that, simultaneously, expresses both GATA3 and RORγT, and produces both Th2 and Th17 typical cytokines (27). These cells showed increased numbers in the circulation of patients with bronchial asthma and may play some role in the...
pathogenesis of this disease. These dual-positive Th2/Th17 cells were found also in the BAL and may characterize a population of patients with severe asthma (28).

Despite these relevant functions of IL-17 and Th17 cells in the pathophysiology of asthma (it should be noted that neutrophil-stimulating factors may also be produced by the epithelium), the results of clinical trials with monoclonal antibodies targeting the IL-17 pathway produced disappointing results, and the development of these drugs for asthma has largely been abandoned (although approved and commercialized for psoriasis, for example): brodalumab, a human anti-IL-17RA immunoglobulin G2 (IgG2) monoclonal antibody reached phase II trials, but did not produce significant effects in asthma, except for a group of patients with high reversibility (29); also secukinumab, an anti-IL17A monoclonal antibody, was discontinued for asthma. Considering the heterogenous pathophysiology (endotypes) of asthma patients, further studies may try and identify particular groups of asthma patients better responding to IL-17 blockade (30). Currently, some phase II trials with monoclonal antibodies targeting Th17 cytokines are under development (e.g., anti-Interleukin-23 subunit p19 inhibitor, anti-IL-17A).

**Th9 cells**

Th9 cells are induced by the concomitant presence of IL-4 and TGF-β during CD4+ T cell activation and are characterized by the production of high amounts of IL-9 (31). IL-9 enhances Th2 cytokine production, airway mucus production, and eosinophil, basophil and mast cell differentiation, among other functions. Although STAT6, IRF4 and BATF are required for IL-9 production, no lineage specific transcription factor has been identified as a "master regulator" of the Th9 phenotype. The expression of IL-9 and IL-9 receptor is increased in bronchial biopsies of patients with atopic asthma (32). Th9-related genes (for example, IL4RA, STAT6, IL9, IL9R, SMAD3, IL33, IL1RL1) have been linked to the development of asthma in humans, and allergic patients have elevated IL-9 producing T cell numbers (31). Several experiments in mouse models indicate that Th9 cells are critical to generate allergic lung inflammation (32). Taking these data into consideration, enokizumab (also known as MEDI-528), a humanized IL-9 neutralizing antibody, was tested in asthmatic patients. Preliminary studies with Enokizumab showed acceptable safety profile and findings suggestive of clinical efficacy in adult patients with mild to moderate asthma (33). However, another study with Enokizumab in adult patients with uncontrolled moderate-to-severe asthma failed to show any benefits in quality of life, asthma exacerbation rates, or FEV1 values (34), and the drug development for asthma was discontinued.

**Follicular helper T cells**

Follicular helper T cells (Tfh) are an independent subset of CD4+ T cells that is specialized in helping B cells. Tfh are characterized by the expression of CXCR5, PD-1, BCL6, BTLA4, ICOS and SAP. Bcl6 is a transcription factor required for Tfh cell differentiation and the absence of Bcl6 expression on T cells impedes germinal center formation and B cell responses to protein antigens (35, 36). Tfh may be a particular differentiation of CD4+ T cells that is plastically acquired when these cells enter the germinal centre (35). Tfh cells were shown to be essential for IgE production (37) and also to be required for the development of allergen specific sensitization and allergic asthma (38). These murine studies proposed that IL-4-committed Tfh cells were the precursors to pathogenic Th2 cells in allergic airway disease (39). Also in humans, Tfh cell numbers correlated with the total IgE blood level, and Tfh cells from asthmatic patients could stimulate IgE production ex vivo in an IL-4-dependent manner (39).

**Regulatory CD4+ T cells**

Regulatory T cells (Tregs) are a subset of CD4+ T cells that suppress or modulate other immune cells to prevent autoimmunity, uncontrolled inflammation, and also allergy. "Natural" CD4+ CD25+ Tregs (nTregs) are produced in the thymus, express the transcription factor Foxp3, and their TCRs have intermediate affinities for self-antigens, allowing them to prevent autoimmunity. Peripherally "induced" Treg cells (iTregs) can be differentiated from naïve Th0 cells of any specificity and are believed to be important for tolerance induction to non-self-antigens, including allergens. Two major groups of iTregs are considered: Foxp3+ iTregs (induced by TGF-β) and Tr1 cells (induced by IL-10). Early studies have shown that depletion of CD4+CD25+ Tregs (here including nTregs and iTregs) results in increased neutrophil and T cell recruitment in the Airways of the mice, increased IL-4 and IL-5 production, and airway hyperreactivity (40). Importantly, Foxp3 deficiency in humans (IPEX syndrome) and mice (Scurfy) causes rapidly fatal immune dysregulation that includes autoimmunity but also allergic manifestations (41). Adoptive cell transfer studies in mice showed that also Tr1 cells can inhibit Th2 and IgE responses in vivo (42). Altogether, Tregs seem to have important roles in allergy and asthma development (these roles have been reviewed elsewhere (7, 43–46)). Tregs may be an important target also for therapeutic reasons. As described before, a great effort has been placed in understanding and targeting IgE, Th2 cytokines and eosinophils to control asthma inflammation. Comparatively, therapeutic strategies eliciting Tregs and immune tolerance induction to control asthma inflammation have been relatively scarce and remain far from clinical use (7). This possibility of inducing Tregs and tolerance for asthma treatment was shown in mouse models of allergic asthma using CD4 co-receptor blockade: antigen-specific tolerance was achieved and it could prevent airway hyperreactivity and eosinophilia, while at the same time maintaining immune competence to mount Th2 responses to unrelated antigens (47).
T cell subsets in asthma

αβ CD8+ T cells

While the fundamental role of CD4+ T cells in the physiopathology of asthma is undisputed, there is recent evidence indicating that also CD8+ T cells may participate, either as a direct interventional or as helpers for CD4+ T cells (48). Clinical studies examining a potential role for CD8+ T cells in asthma hinted to a positive correlation with disease severity (49, 50). The decline of FEV1, even if mild, correlated, both at baseline and follow-up, with the number of CD8+ T cells in the airways (49). Also wheezy infants have elevated numbers of CD8+ T cells in bronchoalveolar lavage (BAL) in comparison to controls, even in the absence of viral infections (51). The specific functions CD8+ T cells in the airways are, however, incompletely understood.

Similar to CD4+ T cells, CD8+ T cells can be polarized into functional subtypes depending on environmental stimuli: Tc1 cells that produce IFN-γ and Tc2 cells producing IL-4, IL-5 and IL-13. A vast array of other subtypes have been proposed, such as, Tc9, Tc17, γδ CD8+ cells and also CD8+ regulatory T cells (52, 53).

Tc1 cells may function both as “friend or foe” in asthma physiopathology. These cells play a major role in defending against viral infections (mainly rhinovirus) with the production of IFN-γ, therefore reducing some viral induced asthma exacerbations. On the other hand, Tc1 cells may suppress or exacerbate pulmonary inflammatory response to allergens, depending on the temporal relationship with the progression of allergic sensitization: in mice models, depletion of Tc1-cells prior to systemic ovalbumin sensitization reduced airway hyperresponsiveness (AHR), lung eosinophilic infiltration and IL-5 production at the lymph nodes of the airway (52). Conversely, when cell depletion occurred subsequent to the initial allergen sensitization, the inflammatory responses were potentiated (54, 55). Both Tc1 and Tc2-cells were linked to suppression of allergic asthma and indirect inhibition of IgE production by stimulation of Th0 differentiation into Th1 cells and subsequent production of IFN-γ (56).

Tc2 cells have been found to exacerbate asthma by secretion of high levels of IL-4, IL-5 and IL-13, in a similar manner to Th2 cells (57). The activity of these cells seems to be more focused in the lung tissue in opposition to lymph nodes (58). In opposition to Tc1, Tc2 and CD8+ Tregs, the subtypes Tc9 and Tc17 cells have low cytotoxic activity (59). When combined with Th2 cells, Tc9 induced key features of asthma, such as increased eosinophil numbers in BAL and elevated lung inflammatory score (48). Tc17 produce IL-17, which was shown to be proinflammatory in pulmonary pathology (60). There is a growing body of evidence to suggest that γδ CD8+ cells may be influential in the regulation of airway inflammation by reducing late-phase AHR and airway eosinophilia via an IFN-γ dependent pathway (61). The role of NK-like CD8+ T cells has been reviewed elsewhere (62).

In conclusion, even though CD8+ T cells appear to play a significant role in asthma, the available evidence is conflicting. There are convincing studies that support their beneficial influence during the initial sensitization to the allergen. On the other hand, it has been suggested that these cells may help propagate the chronic inflammation associated with asthma. The better understanding of the subset diversification of CD8+ T cells will be essential in establishing their functional roles in asthma.

γδ T cells

γδ T cells are a subset of CD4CD8- T cells that express an alternative TCR, with γ and δ chains, as opposed to the classical αβ TCR found on most CD4+ and CD8+ T cells (63). γδ T cells represent a small proportion of peripheral blood T cells (less than 10%) but comprise up to 50% of the T cells within epithelium or mucosa-rich tissues, and these are often composed by oligoclonal subpopulations sharing the same TCR chains. γδ T cells were shown to be involved in several mucosal-related pathologies, namely in the gut. In striking contrast to MHC-restricted αβ T cells, γδ T cells recognize a range of antigens without the presence of MHC molecules. Antigens recognized by these cells are largely unknown, but may include phosphorylated microbial metabolites, markers of cellular stress, and also lipid antigens presented by CD1 molecules, in particular CD1d (63).

γδ T cells expressing Th2-type cytokines were reported in BAL fluid after allergen challenge of asthmatic patients. Also, during exacerbations, asthma patients showed increased proportion of γδ T cells and, ex-vivo, these cells had increased expression of intracellular TNF-α, IL-4 and IL-10 after stimulation in asthmatic vs controls (64). On the other hand, early studies using murine models have shown that airway reactivity is increased in the absence of γδ T cells (65), suggesting a suppressive role of these cells in airway hyperreactivity. In humans, γδ T cell numbers are greater in asthmatic airways during rhinovirus infection and correlate with clinical illness severity, virus load, and airways inflammation (66).

It is important to note that, similar to αβ T cells, γδ T cells are capable of assuming different cytokine production profiles (63) and therefore may play different and opposing roles in asthma, some of which may have contributed to the inconsistent findings on γδ T cells in asthma studies. Also, the localization and TCR specificity may influence γδ T cell roles (46).

Invariant NKT cells

NKT cells are a subset of T cells that coexpress an αβ T cell receptor but also a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells possess an invariant Va24Ja18 TCR and therefore present restricted variability (and are, therefore, also called invariant NKT cells). The most important ligands for NKT cells are glycolipids, in particular α-galactosylceramide (α-GalCer), which is exclusively presented by the MHC class I-like molecule CD1d. NKT cells are capable of producing high amounts of several cytokines upon
activation, with similar variety of cytokines to conventional CD4+ T cells, including IFN-γ but also IL-4, IL-5 and IL-13. The role of NKT cells in allergy and asthma is still controversial (reviewed in (67)). Some studies suggested a suppressive role of NKT, e.g., α-GalCer-stimulated NKT cells suppressed allergic inflammation of the airways by promoting IFN-γ production (68). However, others have found a high proportion of NKT cells among the CD4+ T cells in the lung of asthmatic patients (69). A recent study on the role of NKT cells in asthma was described (71). Interestingly, NKT cells were shown to contribute to asthma development and symptoms, including some unconventional T cells. It is important to remember that a certain amount of plasticity occurs between T cell polarization and multiple cytokines can be produced in response to a diversity of stimuli by the same cell. Therefore, it is perhaps not surprising that some T cells that do not adhere to conventional Th2-low asthma. This may indicate that the absence of Th2 markers does not characterize a “unified” non-Th2 endotype.

Conclusions

Classically, asthma has been considered a Th2 disease. The role of Th2 cells and Th2 associated cytokines is undisputed in the pathophysiology of asthma, in particular in allergic asthma. Therapeutic agents targeting Th2 cytokines and cells have shown great benefits in patients with moderate-to-severe Th2-high asthma. However, it is now clear that asthma is a heterogeneous disease. Furthermore, the division of asthma into only two clinical forms (Th2-high vs Th2-low) is probably an oversimplification. Other types of T cells were shown to contribute to asthma development and symptoms, including some unconventional T cells. It is important to remember that a certain amount of plasticity occurs between T cell polarization and multiple cytokines can be produced in response to a diversity of stimuli by the same cell. Therefore, it is perhaps not surprising that some T cells that do not adhere to conventional types can be found in the lungs of asthmatic patients. Finally, no targeted therapies have been shown to be efficacious for Th2-low asthma. This may indicate that the absence of Th2 markers does not characterize a “unified” non-Th2 endotype.

Conflict of interests

Frederico S. Regateiro received speaker and consultancy fees from AstraZeneca, Novartis, TEVA, Sanofi and Lusomedica-menta, all of which outside the submitted work.

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Cypress pollen allergy in Milan: the story of an ongoing growth

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Introduction
Cypress pollen is one of the most important sources of seasonal respiratory allergy in southern Europe for many years (1). In older epidemiological studies, this type of pollen allergy seemed to be almost exclusive of Spain, Southern France, and of specific Italian regions such as Tuscany, Liguria, Apulia and Campania (1, 2). However, the increasing use of some Cupressaceae species (e.g., Cupressus arizonica) for gardening and reforestation along with the worldwide increase in temperatures has led to a growth of the epidemiological impact of pollinosis induced by this plant species, due both to a higher pollen load and an elongation of the specific pollen season (3). In effect, an Italian multicenter study performed in 2014 (4) found an unexpectedly widespread diffusion of cypress pollen allergy throughout the country, although this was markedly prevalent in the central and southern regions. Milan is the largest northern Italian town, sited in the center of the Po valley basin and has been characterized by a typical continental climate ever since. Until 30 years ago, cypress pollen was considered a negligible allergen source in Lombardy, the region of Milan (5). However, in recent years, cypress pollen allergy has gradually become an increasingly relevant cause of respiratory symptoms during the first few months of the year in a growing proportion of pollen allergic subjects. The present study reports the trends of cypress pollen allergy in a suburban area located just 5 kilometers north of Milan during 15 years of observation.

Methods

Patients
All patients presenting spontaneously for suspect respiratory allergy at the outpatient allergy center of the Clinica San Carlo, Paderno Dugnano from the beginning 2003 to the end of 2017 were considered eligible for the study. Patients were thoroughly interviewed to ascertain which kind of symptoms they complained for (either rhinoconjunctivis, with or without bronchial
asthma) and their seasonality, with a particular focus on the period of cypress pollen allergy in this area (average from mid-end December to the end of March). All patients underwent skin prick tests (SPT) with a series of commercial extracts of airborne allergens (Allergopharma, Reinbeck, Germany), including grass, mugwort, ragweed, pellitory, plantain, birch, olive, plane, and cypress (Cupressus arizonica) as seasonal allergens and Alternaria, house dust mites, dog and cat dander as perennial allergens. Skin tests were carried out following established methods (6); readings were taken after 15 minutes, and skin responses were considered positive in the presence of a wheal and flare reaction exceeding 3 mm in diameter. Histamine 10 mg/mL and saline were used as positive and negative controls, respectively.

Sensitivity to cypress pollen was defined as a positive SPT in the absence of respiratory symptoms in the specific pollen season. In patients sensitized to >3 seasonal allergenic sources including cypress, due to the risk of a false positive result caused by co-recognition of a pollen panallergen such as profilin or polcalcin (7), primary cypress pollen reactivity was confirmed by measuring IgE specific to Cup a 1 by ImmunoCAP (ThermoFisher, Upsala, Sweden). In order to improve the specificity of the test, IgE levels exceeding 0.35 kU/L were considered positive.

Clinical allergy to cypress was defined as the presence of symptoms or rhino-conjunctivitis with or without asthma in the specific pollen season in a patient showing a positive SPT with cypress pollen or IgE specific for Cup a 1 (see above). In the presence of co-sensitization to perennial allergens such as house dust mite, animal dander, or molds the patient was considered as clinically allergic to cypress pollen only if he/she reported a marked increase in symptom severity during the specific cypress pollen season. Monosensitization to cypress pollen was defined as hypersensitivity to cypress pollen in the absence of sensitization to any other seasonal allergen source.

Follow-up study
All patients spontaneously presenting for a control visit at the allergy center at least 4 years after the first visit and who did not score positive for cypress pollen on the initial visit were considered eligible for the follow-up study. These patients underwent SPT with the whole panel of airborne allergens previously described, looking for de-novo cypress sensitization/allergy following the same criteria reported before.

Cypress pollen exposure
Cypress pollen data were provided by the UOC Igiene e Sanità Pubblica Milano Ovest. Pollen counts were performed according to the CEN standard methods (8). Daily average pollen concentrations were expressed as particles per cubic meter of air (p/m³). Observation period ranged between 1995 and 2017; within this period, the Annual Pollen Integral (API) (9), was examined.

Statistics
Cypress pollen sensitization was calculated as a percentage of all subjects sensitized to any pollen source. Cypress pollen allergy was calculated both as a percentage of all subjects sensitized to any pollen source and as a percentage of cypress pollen sensitized patients. The percentage of patients monosensitized to cypress pollen was calculated as well.

Results
Results are summarized in table 1. In total 5626 patients were diagnosed as having pollen allergy at the outpatient clinic between the beginning of 2003 and the end of 2017. Of these, 1125 (20%) were found to be sensitized to cypress pollen. Two-hundred eighty-nine (26% of cypress pollen sensitized subjects; 5% of the whole population) were diagnosed as having frank cypress allergy. Figure 1 shows the trends of cypress sensitization and clinical allergy over time. Both sensitization and allergy showed a gradual increase. Sensitization rate increased from about 15% during the first years of observation to about 25% during the last years of the study period. Similarly, cypress allergy increased from about 3% to about 10%. The linear trend line for both sensitization and allergy showed an almost parallel pattern (figure 1). Figure 2 shows the prevalence of cypress pollen allergy over sensitization during the study period. The linear trend shows a much steeper increase towards the occurrence of allergy among sensitized subjects.

Although altogether 26% of cypress pollen-sensitized subjects were clinically allergic, such proportion showed a gradual increase up to 40% during the last years of the survey.

In contrast, mono-sensitization to cypress pollen remained quite stable over time, ranging between 0% and 9% of cypress pollen sensitized population (table I).

Follow-up data
In total 291 patients underwent SPT during a control visit at least 4 years after the first visit at this center. New sensitizations to cypress pollen were detected in 51/291 (18%) cases, a proportion that is very similar to that of the general study population during the last decade. One third of them (17/51, 33%) reported symptoms suggesting the occurrence of clinical allergy to cypress pollen. New cypress pollen sensitization showed a dramatic increase up from 2010 (table II and figure 3).

Cypress pollen exposure
Figure 4 shows the cypress pollen load in this area from 1995 to 2007. After a period of stability at low level between 1995 and 2000 (median API 3793 Pollen × day/m³) a dramatic increase in mean API was recorded in 2001 followed by a high-level stabilization onwards (median 2001-2017 API: 8705 Pollen × day/m³).
Discussion

Although the present study does not have a strict epidemiological value, as it was not carried out on the general population, it nonetheless shows that both cypress pollen sensitization and allergy are gradually on the rise in the north of Italy. One limit might be the relatively small study population both in the year by year analysis of trends and in the follow-up part of the study but one strength is that the data came from a single geographical area, and were collected using the same methodology throughout the years by one single operator. As short as 30 years ago, cypress pollen was still considered a negligible allergen source in this area (5). Nowadays about one fourth of patients with pollen allergy presenting at this allergy center are sensitized to cypress pollen. During the last decade, there has been an impressive increase in poly-sensitizations to airborne allergens, in many cases due to the sensitization to pollen pan-allergens (profilin, polcalcin) (10). However, the increase in cypress pollen sensitization observed in the present study cannot be ascribed to plant panallergen co-recognition, as patients showing multiple skin reactivity to seasonal airborne allergens, in many cases due to the sensitization to pollen pan-allergens (profilin, polcalcin) (10). However, the increase in cypress pollen sensitization observed in the present study cannot be ascribed to plant panallergen co-recognition, as patients showing multiple skin reactivity to seasonal airborne allergens underwent in vitro component-resolved diagnosis and were included into the cases series only in the presence of Cup a 1 IgE reactivity. Further, since cypress pollen extracts for SPT seem to contain little or no pollen panallergens (7, 11), patients scoring positive on SPT with such extracts are probably primarily sensitized to cypress pollen allergens. Another point is the improved sensitivity of SPT with commercial cypress pollen extracts after the replacement of the formerly employed Cupressus sempervirens with the much more allergenic Cupressus arizonica as source material (12). The main reason for this impressive increase is probably the much heavier cypress pollen burden. In fact, in this geographic area the median annual cypress pollen index more than doubled up from the beginning of the new millennium. This observation is in keeping both with the significant uptrend of the number of days with cypress pollen concentrations exceeding 1 p/m³ in the surroundings of Milan (13), and with previous studies performed in Japan where the policy of planting millions of cedar trees between the early 1950s and early 1970s eventually resulted in a dramatic increase in the prevalence of specific pollen sensitization and allergy (14). In our study, an interesting coincidence of high pollen burden, and marked increase in the prevalence of cypress sensitization both in the general population and in the follow-up group occurred in the years 2010-2012. This period was followed by a period of more moderate pollen burden (2014-2017) associated with a reduction of the percentage of sensitized patients detected. Interestingly, the increase in cypress pollen sensitization was followed by a marked increase in its clinical expression over the years, which reached about 40% at the end of the study period. This finding also is probably the consequence of the increasing cypress pollen burden in this area in recent years. The increasing relevance of cypress pollen allergy in this area has been associated with a marked increase in the number and proportion of patients prescribed cypress pollen immunotherapy over the years (data not shown). The massive increase of cypress pollen in this area could be due to the more frequent planting of cypress trees, particularly in private gardens, for ornamental purposes. It could also be one further indirect consequence of the ongoing global warming trend,
as showed by Ziska et al. (15) for airborne allergenic pollen abundance and seasonality across the northern hemisphere. Indeed they found that the ongoing increase in temperature extremes (T_min and T_max) might already be contributing to extended seasonal duration and increased pollen load for multiple aeroallergenic pollen taxa in diverse locations across the northern hemisphere.

Table I - Cypress pollen sensitivity and allergy in Milan 2003-2017.

<table>
<thead>
<tr>
<th>Year</th>
<th>N° patients</th>
<th>Sensitized %</th>
<th>Allergic %</th>
<th>Monosenitized %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>111</td>
<td>13</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>2004</td>
<td>155</td>
<td>22</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>2005</td>
<td>343</td>
<td>60</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>2006</td>
<td>507</td>
<td>76</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2007</td>
<td>452</td>
<td>59</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>2008</td>
<td>346</td>
<td>51</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>2009</td>
<td>358</td>
<td>69</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>2010</td>
<td>426</td>
<td>100</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>2011</td>
<td>434</td>
<td>121</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>2012</td>
<td>342</td>
<td>99</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>2013</td>
<td>479</td>
<td>111</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>2014</td>
<td>445</td>
<td>82</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>2015</td>
<td>412</td>
<td>76</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>2016</td>
<td>433</td>
<td>94</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>2017</td>
<td>383</td>
<td>94</td>
<td>25</td>
<td>38</td>
</tr>
</tbody>
</table>

Table II - Detection of de-novo cypress pollen sensitization during follow-up visits.

<table>
<thead>
<tr>
<th>Year of control visit</th>
<th>No. follow-up visits</th>
<th>No. patients scoring positive for cypress pollen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>11</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2008</td>
<td>11</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>2009</td>
<td>25</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>2010</td>
<td>33</td>
<td>10 (30%)</td>
</tr>
<tr>
<td>2011</td>
<td>40</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>2012</td>
<td>29</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>2013</td>
<td>50</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>2014</td>
<td>28</td>
<td>5 (18%)</td>
</tr>
<tr>
<td>2015</td>
<td>25</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>2016</td>
<td>21</td>
<td>6 (28%)</td>
</tr>
<tr>
<td>2017</td>
<td>18</td>
<td>3 (18%)</td>
</tr>
</tbody>
</table>

Specific future studies might assess the relationship between climate change and cypress pollen production in the area.

Conclusions

In conclusion, nowadays cypress pollen represents a relevant source of sensitization and seasonal respiratory allergy in this area. This is possibly one further indirect consequence of the global warming trends.

Conflict of interests

The authors declare that they have no conflict of interests.

Figure 3 - Proportion of new sensitizations to cypress pollen in patients undergoing SPT > 3 years after the first visit.

Figure 4 - Annual cypress pollen index (API) in the area north of Milan from 1995 to 2017.
References

Cross-cultural validation of the Portuguese from Portugal version of the Test for Respiratory and Asthma Control in Kids (TRACK) questionnaire

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Key words
TRACK; questionnaire; asthma control; preschool; validation.

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Summary
Introduction. The Test for Respiratory and Asthma Control in Kids (TRACK) is a tool to assess asthma control in preschool children. This study aims to validate the Portuguese from Portugal version of the TRACK questionnaire. Methods. A prospective cohort study was carried out to assess their psychometric characteristics. Caregivers of 141 children under age 5 with asthma symptoms were enrolled. Results. Internal reliability was close to 0.70 (Cronbach’s α). The test-retest reliability was 0.87. TRACK scores were different between well, partially, and non-controlled asthma groups (p < 0.001). Patients rated as having better control showed an increase in TRACK scores. Conclusions. The Portuguese version of the TRACK questionnaire is accurate and reliable for monitoring asthma control. Its use may help to overcome challenges with the management of this age group.

Impact statement
This version of the TRACK questionnaire showed to be valid for use in Portugal as an accurate and reliable tool to monitor asthma control in preschool children.

Introduction
Asthma is a common chronic disease affecting around 6.5% of preschool children in Portugal (1), and accounts for high morbidity and costs, particularly when it becomes uncontrolled (2). Moreover, young children are more prone to develop exacerbations and have less favourable responses to asthma treatments (3, 4) than older children.

According to current guidelines, the goal of the treatment is to achieve and maintain asthma control (5). Moreover, Global INitiative for Asthma (GINA) criteria have been accepted as the standard for physicians assessing asthma control. Questionnaires provide a standardized evaluation of asthma control perception and its use in clinical practice may easily help clinicians to identify uncontrolled preschool asthmatic children (6).
The Test for Respiratory and Asthma Control in Kids (TRACK) is a validated questionnaire that determines respiratory control in children younger than five years of age with symptoms consistent with asthma (7-10). Furthermore, TRACK encompasses both impairment and risk domains assessment, unlike most of the childhood asthma control questionnaires that only capture parent-reported impairment (7).

This questionnaire was already translated and validated into other languages (11-13), including Brazilian Portuguese (14), supporting a wider use across the world. Considering the recognized cultural-linguistic differences between Portuguese from Brazil and Portugal, this study aimed to translate and validate the Portuguese from Portugal version of the TRACK questionnaire.

Materials and methods

Study design
An observational, prospective cohort study was conducted at the Allergy and Immunology Department of Dona Estefânia Hospital (Lisbon, Portugal). The study comprised two visits with an interval of two to six weeks, during the period September 2017 through June 2018, and took into account the COSMIN checklist (15).

Study population
Children younger than five years old, with Portuguese-speaking parents, and with a medical diagnosis of asthma or with symptoms consistent with asthma, were eligible after their parents’ consent. Inclusion criteria were either a history of having two or more episodes of wheezing, shortness of breath or cough that lasted more than 24 hours, or improvement of respiratory symptoms with the use of an aerosolized bronchodilator. Children with respiratory diseases other than asthma or with other chronic disorders or congenital abnormalities were excluded from the study.

TRACK Questionnaire
The TRACK is a five-item caregiver-completed questionnaire that assesses asthma control in children under the age of five. The first four items evaluate impairment based on the frequency of respiratory symptoms, waking up at night because of these symptoms, activity limitation in the last four weeks, and frequency of rescue medication use in the last three months. The fifth item addresses the risk domain, such as the frequency of oral corticosteroid (OCS) use over the past year.

Items may be scored between 0 to 20, and the total score for the whole questionnaire ranges from 0 to 100 points. TRACK higher scores reflecting probably better asthma control and scores lower than 80 suggesting possibly non-controlled asthma (8). There is also evidence supporting that changes in TRACK scores of 10 or more points are representative of the minimal clinically important difference (MCID) (10).

Cultural adaptation of the TRACK questionnaire
The TRACK was adapted to a Portuguese context according to one of the forward-backward-translation methodologies (15, 16). This included four steps: 1) forward translation: two independent native Portuguese speakers, both fluent in English, performed the translation; 2) reconciliation of the forward translations into one preliminary translation was developed by two independent immunoallergists both fluent in English and Portuguese; 3) backward translation of the reconciled version into English was performed by two fluent in English independent translators, and an independent supervisor comparing the text with the original questionnaire for conceptual equivalence; 4) cognitive debriefing was conducted in 10 patients’ caregivers representing the study population. Any uncertainties were discussed with the physicians through face-to-face interviews.

Data collection
Data regarding socio-demographic (age, children gender, and education of the mother and father) and clinical characteristics were recorded.

At baseline (V1) and follow-up (V2) visits, caregivers completed the Portuguese version of the TRACK. Each caregiver and physician rated the disease control status of every child in the previous four weeks on a visual analog scale (VAS) of 0 to 10 cm (completely controlled to not controlled at all) (17). Physicians also assessed asthma control level as controlled, partially controlled, or poorly controlled, based on the GINA guideline recommendations (5), and recorded the number of asthma exacerbations that needed a course of OCS, emergency visits, or hospitalizations in the last year.

Based on the presence or absence of asthma-like symptoms (episodes of wheezing, shortness of breath, or coughing) in the previous four weeks and last 12 months, the timing of respiratory symptoms was defined as currently symptomatic (episodes in the past four weeks), symptomatic in the recent past (episodes in the past 12 months but not within the past four weeks), or asymptomatic (without symptoms for more than 12 months). Therapeutic adjustments were performed at V2 and registered according to the three categories of possible decisions: step-up, no change, or step-down of the therapy level.

The local ethics committee of Centro Hospitalar Universitário de Lisboa Central approved the study. Written informed consent was obtained from each caregiver.
**Statistical analysis**

To calculate the required number of subjects for this study where reliability is measured, a kappa for the null hypothesis of 0.5, a kappa for the alternative hypothesis of 0.7, a statistical significance level of 0.05 (two-sided test), and a power of 80% were considered. A sample size of 124 patients was obtained (18). Categorical variables were described as absolute frequencies and percentages. Continuous variables were presented as median and inter-quartile range (IQR: P25-P75).

Internal consistency of the TRACK was assessed by estimating Cronbach’s alpha coefficient with the responses given by all the parents/caregivers at baseline and follow-up visits. A Cronbach’s alpha coefficient estimate ≥ 0.7 was considered acceptable. The test-retest reliability of the TRACK was evaluated through the intra-class correlation coefficient (ICC) and Lin’s concordance correlation coefficient. Additionally, Bland and Altman plot of the TRACK scores between visits was constructed. For this analysis, only symptomatic in the recent past or asymptomatic patients that remained clinically stable in the follow-up visit 2 to 4 weeks later, were considered.

The criterion validity was assessed by comparing TRACK scores among patient subsets differing on the level of the GINA asthma control (controlled, partially controlled, and non-controlled asthma). Additionally, the screening accuracy of TRACK was evaluated using the receiver operating characteristic (ROC) curve, assuming the hypothesis that higher scores increased the odds of having controlled asthma. It was also calculated the percentage of correctly classified, using 80 as the TRACK cut-off point.

The construct validity was studied by comparing TRACK scores across the three categories of the timing of respiratory symptoms (currently symptomatic, symptomatic in the recent past, and asymptomatic), and with the therapeutic decision. Still, regarding construct validity, the discriminative properties of the TRACK were tested by classifying patients into different groups based on the VAS asthma score of caregivers and physicians. As there is no consensus about anchor points that should be used to classify the levels of asthma control, we decided to attribute equal intervals. Thus, patients with a VAS score < 3.3 were considered as having their asthma controlled, patients with a VAS score between 3.3 and 6.6 as partially controlled asthma, and patients with a VAS score > 6.6 as non-controlled asthma. TRACK’s median score of all groups was compared with the Kruskal-Wallis test.

The responsiveness was measured by comparing the median TRACK scores of those patients that changed between the visits in their GINA scores from partially or non-controlled asthma (GINA score ≥ 1) to controlled asthma (GINA score = 0). Spearman’s correlation coefficients between absolute change scores (V2-V1) of TRACK and both VAS asthma scores of caregivers and physicians, were also estimated (15).

**Results**

**Patients sample**

We included 141 of the 148 eligible children. Dropouts were due to personal reasons (two children) and loss to follow-up (five children). The socio-demographic and clinical characteristics are described in **table I**.

The median (IQR) of the TRACK scores of the 141 patients included in the study was 75.0 (65.0-90.0) and 80.0 (65.0-90.0) points at baseline and follow-up visits, respectively. Regarding asthma control assessment, caregivers overrated controlled asthma in comparison with physicians that used GINA criteria (*i.e.*, 62% vs 38%).

### Table I - Characteristics of patients at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>81 (57.4)</td>
</tr>
<tr>
<td>Mean Age, years (SD)</td>
<td>3.67 (1.15)</td>
</tr>
<tr>
<td>Grouped age, n (%)</td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>36 (25.5)</td>
</tr>
<tr>
<td>3-5</td>
<td>105 (74.5)</td>
</tr>
<tr>
<td>Caregiver education*, n (%)</td>
<td></td>
</tr>
<tr>
<td>High school not completed</td>
<td>26 (18.4)</td>
</tr>
<tr>
<td>High school graduate</td>
<td>37 (26.2)</td>
</tr>
<tr>
<td>Undergraduate or graduate degree</td>
<td>60 (42.6)</td>
</tr>
<tr>
<td>Caregiver rated symptoms status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic last 4 weeks</td>
<td>87 (61.7)</td>
</tr>
<tr>
<td>Asymptomatic recent</td>
<td>22 (15.6)</td>
</tr>
<tr>
<td>Asymptomatic &gt; 1 year</td>
<td>32 (22.7)</td>
</tr>
<tr>
<td>Caregiver rated control status**, n (%)</td>
<td></td>
</tr>
<tr>
<td>Controlled</td>
<td>88 (62.4)</td>
</tr>
<tr>
<td>Partially controlled</td>
<td>35 (24.8)</td>
</tr>
<tr>
<td>Non-controlled</td>
<td>18 (12.8)</td>
</tr>
<tr>
<td>GINA Asthma Control, n (%)</td>
<td></td>
</tr>
<tr>
<td>Controlled</td>
<td>54 (38.3)</td>
</tr>
<tr>
<td>Partially controlled</td>
<td>48 (34.0)</td>
</tr>
<tr>
<td>Non-controlled</td>
<td>39 (27.7)</td>
</tr>
<tr>
<td>Asthma exacerbations, n (%)</td>
<td></td>
</tr>
<tr>
<td>Courses OCS (≥ 1)</td>
<td>80 (56.7)</td>
</tr>
<tr>
<td>Emergency visits (≥ 1)</td>
<td>86 (61.0)</td>
</tr>
<tr>
<td>Hospitalization (≥ 1)</td>
<td>9 (6.4)</td>
</tr>
</tbody>
</table>

*Percentages total less than 100 because some caregivers did not answer all the questions; **using a visual analog scale.
Validation of Portuguese from Portugal TRACK Kids test

**Figure 1** - Portuguese version of the Test for Respiratory and Asthma Control in Kids (TRACK). TRACK is a trademark of the AstraZeneca group of companies. © 2009 AstraZeneca LP. All rights reserved 278650 5/09.

**Cross-cultural adaptation**

A final version for the Portuguese population was completed with few adjustments to the questions, adding “SOS” to the quick-relief medication (4th question) and “emergency” for oral corticosteroids (5th question), and the name of the drugs in both questions was also changed for the most used in our country (figure 1).

**Internal consistency**

Cronbach’s alpha for the total TRACK kids’ questionnaire was 0.79 (V1) and 0.69 (V2). The deletion of each item would decrease this coefficient except for the last one that would increase Cronbach’s alpha to 0.81 and 0.73, respectively.

**Test-retest reliability**

The ICC coefficient estimate between visits was 0.76 (95% CI: 0.42-0.90) for stable patients (p < 0.001). The median (IQR) of the TRACK scores at baseline and in the follow-up visits was not significantly different (95.0 (90.0-95.0) vs 95.0 (90.0-100.0), p = 0.225). Lin’s concordance correlation coefficient was 0.56 (95% CI: 0.28–0.84). The Bland and Altman plot shows the agreement of TRACK scores between baseline and follow-up visits (figure 2).

**Criterion validity**

TRACK scores were significantly different among patients with controlled asthma, patients with partially controlled asthma, and patients with non-controlled asthma (table II and figure 3 a, b). Additionally, the TRACK questionnaire’s ability to discriminate between controlled and non-controlled asthma patients at V1 was assessed through the area under the receiver operating characteristic (AUC) curve. An area of 0.90 (95% CI: 0.85-0.95) was obtained, showing an excellent discriminative performance of the TRACK questionnaire. Using TRACK scores established control cut-off (< 80 versus ≥ 80), control status was correctly classified in 80.1% at baseline. For V2, an AUC of 0.87 (95% CI: 0.82-0.93) and control status was correctly classified in 78.7% of the patients.

**Construct validity**

TRACK scores were significantly different between patients classified as currently symptomatic, symptomatic in the recent past, and asymptomatic (70.0 (50.0-80.0) vs 85.0 (78.8-95.0) vs 95.0 (90.0-95.0) at baseline, and (70.0 (51.3-80.0) vs 85.0 (78.8-90.0) vs 95.0 (90.0-100.0) at the follow-up), both p < 0.001. Moreover, asymptomatic patients had the TRACK highest values while currently symptomatic children had the lowest ones. Based on VAS scores, the median (IQR) of the TRACK scores at baseline between children classified by parents and physicians as having controlled vs partially or non-controlled asthma were, most of them, also significantly different (table III).

Comparing the TRACK scores among a decision of “step-up”, “no change” and “step-down” therapy groups, significantly higher scores were found along with the reduction of the therapy (table IV).
Table II - TRACK scores by GINA levels of asthma control at baseline and follow-up.

<table>
<thead>
<tr>
<th>Control rating - GINA criteria</th>
<th>TRACK scores V1</th>
<th>TRACK scores V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled</td>
<td>90.0 (85.0-95.0)</td>
<td>90.0 (85.0-95.0)</td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>54 (38.3)</td>
<td>69 (48.9)</td>
</tr>
<tr>
<td>Partially controlled</td>
<td>75.0 (65.0-80.0)</td>
<td>75.0 (67.5-85.0)</td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>48 (34.0)</td>
<td>37 (26.2)</td>
</tr>
<tr>
<td>Non-controlled</td>
<td>55.0 (40.0-70.0)</td>
<td>55.0 (45.0-70.0)</td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>39 (27.7)</td>
<td>35 (24.8)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Responsiveness
The median TRACK scores at baseline of partially and non-controlled asthmatic children were significantly lower than those obtained in the follow-up visit when asthma became controlled (67.5 (45.0-75.0) vs 85.0 (70.0-86.3), p < 0.001). Spearman’s correlation coefficients’ estimates of TRACK change scores with caregivers and physicians VAS asthma change scores, between follow-up and baseline visits, showed moderate to strong associations, both p < 0.001 (table V).

Discussion
This study provides evidence that Portuguese from Portugal version of the TRACK is a reliable and valid tool for assessing respiratory control problems, as demonstrated by their adequate psychometric properties, comparable to those of the original validation (8). The internal consistency, close to 0.70, increasing when deleting the fifth item from the scale, was considered acceptable. Similar results were reported by other authors (8, 9) because this last item reflects another different dimension of control, the risk instead of impairment. These related two domains may change independently (19), allowing us to accept values lower than the desirable, in the range of 0.60 to 0.69.

Despite the interval of 2-4 weeks between the test-retest, the reliability found was nearly moderate to good (20) for stable patients. Looking at the confidence interval of ICC, this was quite broad, although following the results obtained either on the original (9) as in other languages validation of the TRACK (11-14). Furthermore, common fluctuations of asthma symptoms in preschool children probably were contributing to these findings. Albeit there was no consensus, the GINA criteria were selected as the standard for rating asthma control levels because they are well accepted in clinical and research settings (5-7, 10). Concerning criterion and construct validities, our results were similar to those of the TRACK original validation study (8, 9), with median scores of the TRACK differing significantly in the expected direction for levels of respiratory control based on GINA guidelines, the timing of respiratory symptoms, VAS asthma scores, and recommendations of changes in therapy. Regarding discriminative accuracy, the TRACK showed also good areas under the ROC curve relative to the ratings of asthma control. The AUC values of 0.90 and 0.87 were similar or even better than those reported for the development and validation samples (8). Likewise, the questionnaire correctly classified respiratory control levels in nearly 80% of children (8).
Table III - Evaluation of the discriminative ability of TRACK using asthma VAS scores classification.

<table>
<thead>
<tr>
<th>Control rating - Caregivers VAS score</th>
<th>Controlled</th>
<th>Partially controlled</th>
<th>Non-controlled</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRACK scores V1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>85.0 (75.0-95.0)</td>
<td>65.0 (45.0-80.0)</td>
<td>47.5 (37.5-61.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TRACK scores V2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>85.0 (75.0-95.0)</td>
<td>65.0 (50.0-78.8)</td>
<td>52.5 (35.0-81.3)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Control rating - Physicians VAS score

| TRACK scores V1                      |            |                      |                |         |
| Median (P25-P75) n (%)               | 90.0 (80.0-95.0) | 75.0 (65.0-80.0)  | 45.0 (40.0-65.0) | < 0.001 |
| TRACK scores V2                      |            |                      |                |         |
| Median (P25-P75) n (%)               | 90.0 (80.0-95.0) | 75.0 (63.8-81.3)  | 50.0 (40.0-65.0) | < 0.001 |

Table IV - Evaluation of the discriminative ability of TRACK using a therapeutic decision.

<table>
<thead>
<tr>
<th>Therapeutic recommendation</th>
<th>Step-up</th>
<th>No change</th>
<th>Step-down</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRACK scores V2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (P25-P75) n (%)</td>
<td>60.0 (50.0-75.0)</td>
<td>85.0 (80.0-90.0)</td>
<td>100.0 (91.3-100.0)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Good responsiveness to change in asthma control was shown when we compared median TRACK change scores among the children, grouped by the physicians’ GINA, and corresponding VAS change scores. The results of this comparison suggested a parallel change of TRACK with control status, supporting the discriminant validity of the TRACK scores. Our results were also in agreement with those obtained by Wandalsen et al., in what concerns the discriminatory capacity as the reliability of the Brazilian version of TRACK (14).

Based on these findings, the implementation of the TRACK questionnaire as a tool for assessing asthma control in preschool children may improve asthma management, identifying uncontrolled patients easier. These patients require medication adjustments and consequently, their better identification may lead to significant reductions of the disease burden.

The main limitation of this study was the inclusion of a relatively small number of patients from a single center. Nevertheless, this Portuguese version of the TRACK probably exhibits similar psychometric properties in other clinical settings. Additional studies and more widespread use of TRACK will confirm this expectation.

One of the main strengths of our study is the inclusion in the assessment of the asthma control of other validated tools such as

Table V - Correlation coefficients’ estimates of TRACK with caregivers and physicians VAS change scores between visits (V2-V1).

<table>
<thead>
<tr>
<th>Change of scores</th>
<th>Caregivers VAS* asthma</th>
<th>Physicians VAS* asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRACK</td>
<td>- 0.545</td>
<td>- 0.684</td>
</tr>
<tr>
<td></td>
<td>(- 0.656 - - 0.410)</td>
<td>(- 0.768 - - 0.578)</td>
</tr>
</tbody>
</table>

*VAS: Visual Analog Scale.

VAS scores in addition to the GINA criteria, which overcomes any possible drawback of the selected standard.

Conclusions

In conclusion, the TRACK is a reliable and valid tool to assess asthma control in Portuguese preschool children. The findings of this study are important because asthma is highly prevalent, and the use of the Portuguese version of the TRACK may help clinicians to overcome some difficulties associated with pediatric asthma care.
Acknowledgments

The authors express their gratitude to the participant children, their parents, and caregivers for their essential contribution. The authors thank Daniel Virella MD MSc for the methodological support.

Conflict of interests

The authors declare that they have no conflict of interests.

References


Spiritual well-being and quality of life are impaired in chronic urticaria

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Key words
Angioedema; chronic inducible urticaria; chronic spontaneous urticaria; quality of life; spirituality.

Summary
Background. Patients with chronic urticaria (CU) often report an impaired quality of life (QoL). Although a positive effect of addressing spirituality in health care has been proved in several chronic diseases, its potential role in CU has received no attention. Objective. We aim to evaluate spirituality and QoL in CU subjects. Methods. In a single-centre observational study, 100 CU subjects were investigated using Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being (FACIT-Sp-12) scale, Chronic Urticaria Quality of life Questionnaire (CU-Q2oL) and Urticaria Control Test (UCT). Results. Of 100 subjects, 82 were female and 18 were male. It was observed that subjects with poorly controlled CU presented FACIT Sp-12 meaning/peace (p = 0.004) significantly lower, and CU-Q2oL (p < 0.0001) significantly higher (worst QoL) than subjects with controlled CU. There was no difference in the FACIT Sp-12 faith (p = 0.43) between groups. There was moderate direct correlation between FACIT Sp-12 faith and FACIT Sp-12 meaning/peace (r = 0.483; p < 0.0001; n = 100). There was a significant strong inverse correlation between the CU-Q2oL and the UCT (r = -0.762; p < 0.0001; n = 100). No correlation was found between the FACIT Sp-12 faith and CU-Q2oL, neither with UCT. Conclusions. No study has ever investigated the role of spirituality in managing patients with urticaria. Our findings support the impact of poorly controlled urticaria in spiritual well-being and QoL. Therefore, clinicians should pay more attention to spirituality among CU patients. We suggest that urticaria guidelines should include specific recommendations on spirituality assessment.

Impact statement
The burden of poorly controlled chronic urticaria impaires patients' quality of life and spiritual well-being.

Abbreviations
AAS: Angioedema Activity Score
AE-QoL: Angioedema Quality of Life Questionnaire
ClIndU: Chronic Inducible Urticaria
CSU: Chronic Spontaneous Urticaria
CU: Chronic Urticaria
CU-Q2oL: Chronic Urticaria Quality of life Questionnaire
FACIT-Sp-12: Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being scale
MBS: Mind-Body-Soul
PROs: Patient Reported Outcomes
QoL: Quality of Life
SpWB: Spiritual Well-being
GA2LEN UCARE: Urticaria Center of Reference and Excellence
UCT: Urticaria Control Test
UAS7: Weekly Urticaria Activity Score
Introduction

Chronic urticaria (CU) is a skin disorder in which red, swollen, itchy, and sometimes painful hives (wheals), angioedema, or both, repeatedly occur for more than 6 weeks (1). Prevalence is estimated up to 1% in the general population (2), with those aged between 30 and 50 years most affected, and females affected approximately twice as often as males (3-6). The current guidelines classify CU as spontaneous (chronic spontaneous urticaria (CSU), with no specified eliciting factor involved) or inducible (chronic inducible urticaria (CIndU), with a specific eliciting factor involved) (1). Patients may concurrently experience CSU and CIndU in approximately 20% of cases (4).

Existing evidence indicates that symptoms of CU have a deleterious effect on the quality of life (QoL) (2, 7-9). It impacts daily activities and emotional well-being; some patients’ health status is comparable to that of coronary artery disease and severe asthma patients. It also causes inconvenience in family structures, compromising performance at work, school, and negatively impacting on leisure activities. It compromises patients’ QoL, mainly those with more severe disease or who are diagnosed with chronic spontaneous urticaria (7, 10). Until now there are no reliable biomarkers to identify and measure disease activity in CSU. Consequently, use of patient reported outcomes (PROs) is crucial when evaluating and monitoring different aspects of chronic urticaria such as disease activity/severity, disease control, and QoL. Five different PROs that measure various aspects of disease severity/activity and QoL are used routinely in research and clinical practice of chronic urticaria. Three of these PROs are urticaria-specific: weekly Urticaria Activity Score (UAS7), Urticaria Control Test (UCT), and Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL); and then two for angioedema: Angioedema Activity Score (AAS) and Angioedema Quality of Life Questionnaire (AE-QoL) (11-15).

In 1999, the World Health Organization (WHO) started to describe the QoL as multidimensional, in the physical, psychological, social and spiritual dimensions (16). Among those likely to be important is spiritual well-being (SpWB). Viewed as a multifaceted construct, SpWB usually refers to a sense of meaning or purpose in life, inner peace and harmony, and the strength and comfort drawn from faith (17). SpWB has been measured over two dimensions (Meaning/Peace and Faith). Recent studies suggest a broad protective relationship between religious participation and population health (18). Although a positive effect of addressing spirituality in health care has been proved in several chronic diseases, spiritual well-being in patients with CU has never received attention.

The aim of this study was to evaluate spirituality and QoL in CU subjects with different control levels (subjects with controlled CU and those with poorly controlled CU).

Methods

Patients

We conducted a prospective single-centre observational study with 100 consecutive patients from the outpatient clinic of a Urticaria Center of Reference and Excellence (GA2 LEN UCARE, www.ga2len-ucare.com) (18) at the Immunology Service of a university hospital. Patients were enrolled after informed consent was obtained. The study was submitted and approved by Comitê de Ética em Pesquisa do Hospital Universitário Clementino Fraga Filho (HUCFF-UFRJ), CAAE 45067715.5.0000.5257.

Measures

Urticaria control assessment

The UCT is a developed and validated instrument to determine the level of disease control in all forms of CU. It was originally developed in German and it has been validated to brazilian portuguese by our group. Two forms of the UCT are available: the long form UCT (UCTlg) (8 questions) and the short form UCT (UCTsh) (4 questions). Because the results of both UCT forms have been found to correlate extensively, the more convenient UCTsh is primarily used, both, in clinical trials and routine patient care. The categorizing recommendation is poorly controlled CU (UCT < 12) and well controlled CU (UCT ≥ 12) (12, 15).

Spiritual Well-Being

SpWB was measured using the Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being (FACIT-Sp-12) Questionnaire (20-22). This is a valid and reliable instrument, developed in 1990, to provide an inclusive measure of spirituality in research and clinical practice. It is a self-administered questionnaire that contains twelve, four point Likert scale, closed questions (0 = Not at all, 1 = little bit, 2 = Some-what, 3 = Quite a bit, 4 = Very Much) and 2 subscales in the Brazilian version: meaning/peace (items 1-8) and faith (items 9-12) (22-24) (Figure 1). The total score was obtained by summing all individual items (range, 0-48, with higher scores indicating greater spiritual well-being), and the subscale scores were obtained by summing all items in each domain. The meaning/peace subscale measures sense of meaning, peace and harmony, and purpose in life (range, 0-32). The faith subscale assesses the association between illness, faith, and spiritual beliefs as well as how one finds solace in one’s faith (range, 0-16) (23, 24).

Quality of Life

Quality of life was measured using the CU-Q2oL. The CU-Q2oL is a CSU-specific health-related QoL questionnaire consisting of 23 questions. The questions cover different aspects of CSU’s impact on patients’ lives including pruritus, swelling, daily life activities, sleep, appearance, and limitations. CU-Q2oL scores range from 23 to 115, with a higher score indicating stronger impairment of health-related QoL (13, 14).
SpWB and QoL are impaired in chronic urticaria

Statistical analysis
First, to compare SpWB and QoL between the 2 groups (subjects with controlled CU and those with not controlled CU), univariate analyses were performed using a Mann-Whitney U test. Association between SpWB, QoL and disease control was assessed by Spearman’s rank correlation coefficient. Nonparametric models were performed because the scores had a non-Gaussian distribution, according to the rejection of the hypothesis by the Shapiro-Wilk normality test. A p value of <0.05 was regarded as statistically significant. All statistical analyses were performed using SAS® System statistical software, version 6.11 (SAS Institute, Inc., Cary, North Carolina).

Results
Subject Characteristics
Of 100 subjects, 82 were female and 18 were male (mean ± standard deviation (SD) age, 43 ± 15 years) (table I). Subjects distribution by age range are presented in figure 2, and characteristics of the subjects are summarized in table I.

Score Characteristics
Table II provides a description of the scores, in the total sample and by groups: poorly controlled CU (n = 45) and controlled CU (n = 55). The scores did not present a normal distribution (Gaussian), according to the Shapiro-Wilk normality test, at the level of 5%.

Therefore, the most appropriate measures for summarizing the data were by quartiles (median, interquartile range (Q1-Q3), minimum and maximum).

FACIT-Sp-12 Meaning/Peace, FACIT-Sp-12 Faith and CU-Q2oL between controlled and not controlled subjects
It was observed that subjects with poorly controlled CU presented FACIT Sp-12 meaning/peace (p = 0.004) significantly lower, and CU-Q2oL (p < 0.0001) significantly higher (worst QoL) than subjects with controlled CU. There was no difference in the FACIT Sp-12 faith (p = 0.43) between the groups.

FACIT-Sp-12 Meaning/Peace, FACIT-Sp-12 Faith, CU-Q2oL and UCT correlation
There was moderate direct correlation between FACIT Sp-12 faith and FACIT Sp-12 meaning/peace (r = 0.483; p < 0.0001). Significant moderate inverse correlation was found between FACIT Sp-12 meaning/peace and CU-Q2oL (r = -0.457; p < 0.0001). FACIT-Sp-12 meaning/peace correlated weak with UCT (r = 0.331; p = 0.0007) (figure 3).

No correlation was found between the FACIT Sp-12 faith with UCT (r = 0.055; p = 0.58) (figure 4), neither with CU-Q2oL (r = -0.113; p = 0.26).

There was a significant strong inverse correlation between the CU-Q2oL and the UCT (r = -0.762; p < 0.0001) (figure 5).
Table I - Patient sample characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. of patients</td>
<td>100</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Females (n of patients)</td>
<td>82</td>
</tr>
<tr>
<td>Males (n of patients)</td>
<td>18</td>
</tr>
<tr>
<td>Age ± SD (range), years</td>
<td>43 ± 15, (13-78)</td>
</tr>
<tr>
<td>Types of Chronic Urticaria</td>
<td></td>
</tr>
<tr>
<td>CSU</td>
<td>51</td>
</tr>
<tr>
<td>CSU + CIndU</td>
<td>40</td>
</tr>
<tr>
<td>CIndU</td>
<td>9</td>
</tr>
<tr>
<td>Angioedema</td>
<td>65</td>
</tr>
</tbody>
</table>

SD: Standard deviation; CSU: chronic spontaneous urticaria; CIndU: chronic inducible urticaria.

**Figure 2 - Subjects distribution by age range.**

![Bar chart showing age distribution](image)

**Discussion**

To the best of our knowledge, this is the first study to assess spiritual well-being in subjects with CU. We found that subjects with poorly controlled CU appeared to experience spiritual well-being on a worst level than those with controlled CU. The skin is the largest organ of the human body and seems to be closely related to changes in emotions, psychological state, and spirituality. A very common example is how skin flushing, or pallor reflect some emotional states. Some authors mention that modern understanding of skin disorders and how to treat them have brought important advances, but sometimes treatment is hindered until the spiritual aspect is adequately addressed (25). Our study measured QoL and SpWB concurrently. We found that not controlled CU subjects present negatively associated with SpWB and QoL. This is in accordance with recent research which mostly demonstrated emotional distress affect CU and other skin disease patients QoL, leading them to have a low SpWB (9, 26, 27). Therefore, providing spiritual care might improve QoL among such patients.

In accordance with Brady et al. study on the evaluation of spirituality impact in quality of life of oncology patients we found that the faith subscale evidenced significantly smaller correlations with QoL than did the meaning/peace subscale (28). Faith’s contribution seems to be smaller and not significant, having no impact patients’ life enjoyment despite chronic symptoms. In a recent study which aim was to identify the different aspects of a family member’s QoL that may be affected by having a family member with skin disease, faith was mentioned by a few participants (8%). Interestingly, the father of a patient with atopic eczema said: “my faith in God helps me, it gives me strength, hope and patience” (29).

Meaning/Peace was the best predictor of QoL in the CU patients’ evaluated.

In line with the UCT development study there was a strong correlation between UCT scores and CU-Q2oL (12). In this study we found that UCT scores didn’t well correlate with FAC-IT-Sp-12 subscales, indicating that SpWB were not associated with CU patients’ control status.

Current literature suggests several potential interventions intended to help individuals engage in positive spiritual coping. Life review has been suggested as a potential intervention. This intervention is targeted at helping individuals work through the meaning-making process to achieve a positive view of past and present life events (30). Mindful living and spirituality have been emphasized and promoted by the NIH (31), Mayo Clinic, and the National Psoriasis Foundation as a means to decrease stress and improve quality of life and as an adjunct to pharmacological therapy. The literature indicates some patient-generated suggestions for interventions. Regarding spiritually related needs, some patients with end-stage heart failure suggested that home visits, visits from volunteers, and a supporting attitude from health care providers were important for their well-being (32). Therefore, clinicians should pay more attention to spirituality among CU patients. Meditation, mind-body-soul (MBS) therapies, and yoga seems to improve stress and anxiety levels (33, 34).

**Conclusions**

Spirituality as marked by the meaning of self and inner independence cooperates with the affective states to determine the QoL of patients with CU. Considering patients’ spiritual concerns in the clinical setting is critical in enhancing QoL. No study has
ever investigated the role of spirituality in managing patients with urticaria. Facit-Sp-12 may be a complementary tool to clinical management and evaluation, but this does not substitute CU-Q2oL. Thereby, the clinical management can be done through looking for self-knowledge such as psychological approaches.

Our findings support the impact of poorly controlled urticaria on SpWB and QoL. For many patients, spiritual, existential, or religious beliefs can affect their understanding of illness and can influence treatment decisions. In line with worldwide promotion of patient-centered care, we suggest that urticaria guidelines should include specific recommendations on focusing patients’ spirituality assessment, such as using FACIT-Sp-12 in each medical appointment.

**Ethics**

This research has been submitted and approved by Comitê de Ética em Pesquisa do Hospital Universitário Clementino Fraga Filho (HUCFF-UFRJ), number CAAE 45067715.5.0000.5257.

**Fundings**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Contributors**

Sergio Dortas Junior analyzed data and drafted the manuscript. Guilherme Azizi analyzed data. Rafael Moret, Rossy Bastos Junior and Solange Valle drafted the manuscript. All authors approved the final manuscript.

**Table II - Scores results and comparison between controlled and poorly controlled urticaria groups.**

<table>
<thead>
<tr>
<th>Scores</th>
<th>n</th>
<th>mean</th>
<th>SD</th>
<th>median</th>
<th>IQR</th>
<th>r_s</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facit Sp-12 meaning/peace</td>
<td>100</td>
<td>21.6</td>
<td>4.9</td>
<td>21.5</td>
<td>18 - 24</td>
<td>0.331</td>
<td>0.0007</td>
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<tr>
<td>Facit Sp-12 faith</td>
<td>100</td>
<td>13.1</td>
<td>3.1</td>
<td>14</td>
<td>12 - 16</td>
<td>0.055</td>
<td>0.58</td>
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<td>CU-Q2oL</td>
<td>100</td>
<td>46.8</td>
<td>19.9</td>
<td>44</td>
<td>28 - 58</td>
<td>-0.762</td>
<td>&lt; 0.0001</td>
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<td>UCT</td>
<td>100</td>
<td>10.9</td>
<td>4.7</td>
<td>12</td>
<td>8 - 15</td>
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<td><strong>UCT&lt; 12</strong></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Facit Sp-12 meaning/peace</td>
<td>45</td>
<td>20.0</td>
<td>5.0</td>
<td>19</td>
<td>17.5 - 24</td>
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<tr>
<td>Facit Sp-12 faith</td>
<td>45</td>
<td>12.7</td>
<td>3.5</td>
<td>14</td>
<td>10 - 16</td>
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<tr>
<td>CU-Q2oL</td>
<td>45</td>
<td>62.1</td>
<td>17.8</td>
<td>58</td>
<td>51 - 75</td>
<td></td>
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<tr>
<td>UCT</td>
<td>45</td>
<td>6.8</td>
<td>3.7</td>
<td>8</td>
<td>4 - 11</td>
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<td></td>
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<tr>
<td><strong>UCT ≥ 12</strong></td>
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<tr>
<td>Facit Sp-12 meaning/peace</td>
<td>55</td>
<td>22.9</td>
<td>4.4</td>
<td>23</td>
<td>19 - 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facit Sp-12 faith</td>
<td>55</td>
<td>13.4</td>
<td>2.7</td>
<td>14</td>
<td>12 - 16</td>
<td></td>
<td></td>
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<tr>
<td>CU-Q2oL</td>
<td>55</td>
<td>34.3</td>
<td>10.7</td>
<td>31</td>
<td>26 - 41</td>
<td></td>
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<tr>
<td>UCT</td>
<td>55</td>
<td>14.3</td>
<td>1.6</td>
<td>14</td>
<td>13 - 16</td>
<td></td>
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</tbody>
</table>

SD: Standard deviation; IQR: interquartile range; r_s: Spearman correlation coefficient.
Conflict of interests

Sergio Dortas Junior declares he received lecture fees from Astrazeneca Brazil. Sergio Dortas Junior and Solange Valle declare they received lecture fees from Novartis Brazil. Rossy Bastos Junior and Solange Valle declare they received lecture fees from Takeda Brazil. Guilherme Azizi and Rafael Moret have no conflicts of interest to declare.

References

SpWB and QoL are impaired in chronic urticaria

Which skin prick test wheal size detects true allergy to Salsola kali?

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Summary

Background. Sensitization to Salsola kali (Sk) weed pollen allergen is the most common cause of seasonal allergic rhinitis (SAR) in Middle East countries. Aim. To identify Salsola kali skin prick test (SkSPT) wheal size cut-off, able to determine true allergy among adult patients with moderate to severe SAR, who are in need of Salsola kali allergen specific immunotherapy (SkAIT). Methods. In 151 adults with moderate to severe SAR, mean age 32.79 ± 10.79 years, of both gender (females: 43.05%), with a positive SkSPT, (i.e., cut off wheal longest diameter of 3 mm) and one or more other local weed pollens, Salsola kali nasal provocation test (SkNPT) was carried out. Response was assessed both subjectively, with scores, and objectively, by measuring peak nasal inspiratory flow (PNIF). Safety profile of SkNPT was assessed using peak expiratory flow rate (PEF) measurements. Results. SkNPT positive response was found in 125 patients (82.78%). Mean skin prick test (SPT) wheal size to Sk was bigger in the nasal provocatin test (NPT) positive group (9 mm) compared to the NPT negative patients (5 mm), p < 0.0001. ROC analysis showed that a SPT wheal size to Sk at the threshold of > 7.5 mm enabled identification of SkNPT positivity with a sensitivity of 73.6% and specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; p < 0.0001). Conclusions. SPT wheal size of 3 mm might overestimate the presence of real allergy to Sk in a desert environment. A SPT wheal size > 7.5 mm for Sk appears to distinguish individuals who develop disease from those who does not. Physicians should select the proper SPT wheal size value as an appropriate criterion according to the allergen than using a uniform cut off value in patients eligible for SkAIT.

Impact statement

A SPT wheal size > 7.5 mm for Salsola kali appears to distinguish individuals who develop seasonal allergic rhinitis from those who does not, with a sensitivity of 73.6% and specificity of 100.0%.

Abbreviations

Sk: Salsola kali
SAR: Seasonal Allergic Rhinitis
SPT: Skin Prick Test
SkSPT: Salsola kali Skin Prick Test
NPT: Nasal Provocation Test
SkNPT: Salsola kali Nasal Provocation Test
AIT: Allergen Specific Immunotherapy
SkAIT: Salsola kali Allergen Specific Immunotherapy
PNIF: Peak Nasal Inspiratory Flow
PEF: Peak Expiratory Flow
SssIgE: Serum Specific Immunoglobulin E
ROC: Receiver Operating characteristics
CI: Confidence Interval
CRP: C-reactive protein
TNSS: Total nasal symptom score
TCSS: Total clinical symptom score
Introduction

Despite the scarce vegetation in Kuwait’s desert environment, SAR is one of the most common respiratory allergies (1). Previous studies demonstrated that the prevalence of allergic rhinitis symptoms; ever, current symptoms and physician-diagnosed allergic rhinitis were 43.9%, 30.7%, and 17.1%, respectively (2). Chenopods weed family, including Salsola kali (Sk) are a dominant sensitizing allergen, showing almost always a markedly greater response than all other allergens (3). They are highly allergenic, very invasive and fast-growing in arid salty areas. Maximal level of weed pollen is during March-April and September-October (2). Although more than a hundred genera comprise Chenopods family, it seems that Sk have been mostly associated with clinical symptoms of allergy in Kuwait (3), with oscillation of pollen grain in the atmosphere between 30 and 80 grains/m (4) during peak of the season.

Identification of clinically relevant allergen is the key step for the diagnosis of allergy. The most common diagnostic tools in identifying allergen sensitization is skin prick test (SPT) and an in vitro test to detect serum specific immunoglobulin E (SsIgE). SPT, is a safe and simple procedure (5, 6) and remains a fundamental diagnostic tool in the practice of clinical allergy. Although the cut off for a positive immediate skin reaction of a 3 mm wheal size diameter is a widely accepted criterion (7), there is no consensus among researchers on the diagnostic accuracy of SPT (8, 9) and a 3 mm criterion is not always sufficient for accurate diagnosis of true allergy (10, 11). However, few scientific data are available to evaluate the validity of SPT wheal size criterion (11-13).

Given the high rate of sensitization to Sk in our atopic population (76.7%) (3), we validated the scientific basis of the 3 mm threshold of SPT wheal size for Sk as the key diagnostic tool for allergen specific immunotherapy (AIT) and compared that with Salsola kali nasal provocation test (SkNPT), as a more accurate and specific diagnostic tool (13, 14). Nasal provocation test (NPT) is recommended whenever discrepancies arise or difficulties exist in the assessment of patient’s medical history and results of SPT (15, 16). This is important in avoiding overestimation of true allergy and miscalculation of SkAIT.

The aim of this study was to assess the reliability of SkSPT wheal size in detecting positive SkNPT to determine true allergy in adult patients with SAR, polysensitized, positive to Salsola and one or more allergens from Chenopodiaceae and Amaranthaceae families, eligible for SkAIT.

Patients and methods

In the 151 adult SAR patients referred to Al Rasheed Allergy Centre in Kuwait from September 2017 to February 2018, with a positive SPT to Sk (i.e., cut off wheal longest diameter of 3 mm) and one or more other local weed pollens, in need for SkAIT, nasal provocation test with Sk was carried out. All patients were poly-sensitized to local weeds including Sk. Mild form of SAR, pregnant women, patients with upper respiratory tract infection (confirmed by a normal C-reactive protein, CRP), patient with dermographism and those with significant comorbidities were not included. Furthermore, patients with peak nasal inspiratory flow (PNIF) < 60 L/min, peak expiratory flow (PEF) < 350 L/min, choanal atresia, nasal polyp, septal perforation, atrophic rhinitis, adenoids obstructing nasal ventilation were also not included. All patients were informed about the risk and outcomes of the procedure and provided informed consent. Ethical clearance was granted by Ministry of Health Research Ethics Committee (number 2017/669).

Skin prick test

SPT was used as the gold standard to describe atopic status. SPT was performed by single head prick lancets on the volar aspect of the forearm, 2 to 3 cm from the wrist and the antecubital fossae as recommended (6). We used a battery of indoor and outdoor inhalant allergens (Diater, Spain) which included Sk and other local pollens, from the same family. All patients refrained 7 days from treatment with antihistamines. Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively. Results were read 15–20 minutes following allergen extract application.

Nasal Provocation Test

Bilateral nasal provocation test was done at least 4 weeks after weed pollen season, and 3–4 weeks after upper respiratory tract infection (confirmed by normal CRP value), 1 week after discontinuation of oral antihistamine, nasal corticosteroid, and nasal decongestant, and 2 weeks after antidepressant, or oral corticosteroids (> 10 mg/day). Allergen extract was provided from the same manufacturer as it was for SPT (Diater, Spain). Due to less abundance of other weeds from Amaranthaceae and Chenopodiaceae family in our environment, and our local AIT practice using Sk extract only, nasal provocation with other allergen was not done. Fifteen minutes after accommodation to room temperature and saline nasal provocation, to exclude nasal hyperreactivity, progressively increasing concentrations (0.5 and 5 HEP/mL) of freshly reconstituted, commercial freeze-dried allergen solution (10 IRHEP/mL) were administered in the inferior nasal turbinates intranasal at 20-min intervals in the form of a nasal spray (100 μL/puff). Nasal reaction was assessed following the manufacturer’s recommendations 20 min (pinched nose for 10 min and 10 min un-pinched) after each dose (concentration) of allergen, as follows: sneezing: 0 (0–2 sneezes), 1 (3–4 sneezes), 3 (≥ 5 sneezes); nasal itching, rhinorrhea, and nasal obstruction: 1 (mild), 2 (moderate), 3 (severe); palate, eyes, and/or ears itching: 0 (absent), 1 (present). In the case of a positive response to any concentration, further provocation was interrupted. The provocation outcome was assessed subjectively and objectively in all
patients. A subjective method was based on patient’s assessment, expressed as a sum of symptoms; total nasal symptom score (TNSS), and a positive score was if the sum ≥ 5 of the maximal 15. Peak nasal inspiratory flow (PNIF) measurement served as objective assessment of SkNPT outcome, while peak expiratory flow (PEF) was used as a safety control. Three PNIF and PEF measurements were taken; before challenge (basal value), 20 min after placebo (saline), after each given allergen concentration and 8 hours after the challenge. The best of the three PNIF and PEF measurements at each time point was recorded. Reduction in PNIF ≥ 20%, compared to a baseline value, was an objective measure of nasal patency. A reduction in PEF ≤ 20% excluded the involvement of the lower airways during the procedure. A positive NPT was considered when we had both a positive TNSS and a reduction of PNIF ≥ 20% compared to a baseline value. A device (Clement-Clark Int. Ltd., Harlow, UK) was used for both PNIF and PEF measurements.

**Statistic**

Accuracy and normality were determined using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Non-parametric and parametric methods were used to calculate statistical significance. Student’s t test, the Mann-Whitney U test, Fisher’s test, and the χ² test were used to calculate the differences between groups. ANOVA was used to calculate the relative difference distribution variance between variables. Receiver operating characteristics (ROC) analysis was used to determine the optimum value of the SPT wheal size predictive score, and the Hanley and McNeil methods were used to calculate the area under the curve. The statistical hypotheses were tested at the level of α = 0.05, and the difference between the groups in the sample was considered significant with two-sided p < 0.05. Statistical significance was considered to be achieved at p < 0.05, p < 0.01, and p < 0.001. All data was analysed using GraphPad Prism 7 (San Diego, CA, USA).

**Results**

Total of 151 patients, sensitized to Sk as well as to other local weed pollens from the same less abundant weed family in Kuwait, were included. The mean age was 32.79 ± 10.79 years; females: 65 (43.05%), with median total clinical symptom score (TCSS) of 12 (minimum 9 and maximum 15). SkNPT was positive in 125 (82.78%) patients, while 17.22% did not react. The mean wheal size was significantly bigger in the challenge positive group when compared with challenge negative patients (median; minimum; maximum: 9; 3; 19 vs 5; 3; 7; challenge positive, negative patients, respectively, p < 0.0001) (table I). In addition to the subjective assessment of SkNPT using TNSS, positivity was proven by a reduction of PNIF value during procedure. A significant reduction in PNIF, in patients with a positive challenge response,

<table>
<thead>
<tr>
<th>Table I - Patients’ baseline and follow up characteristics.</th>
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<tbody>
<tr>
<td>Patients; number</td>
</tr>
<tr>
<td>Females; number (%)</td>
</tr>
<tr>
<td>Age (years) (mean ± standard deviation)</td>
</tr>
<tr>
<td>Total Clinical Symptom Score (TCSS) (median [minimum, maximum])</td>
</tr>
<tr>
<td>SkNPT mean wheal size (mm) (median [minimum, maximum])</td>
</tr>
<tr>
<td>SkNPT positive; number (%)</td>
</tr>
<tr>
<td>SPT mean wheal size (mm) (median [minimum, maximum])</td>
</tr>
<tr>
<td>PNIF (mean ± standard deviation)</td>
</tr>
<tr>
<td>PNIF fall after SkNPT (median [minimum, maximum])</td>
</tr>
<tr>
<td>PEF (mean ± standard deviation)</td>
</tr>
<tr>
<td>PEF fall after SkNPT (median [minimum, maximum])</td>
</tr>
</tbody>
</table>
was detected (96.23 ± 22.23; 71.75 ± 19.39; basal PNIF; PNIF during procedure, respectively). Furthermore, its recovering to the baseline value 8 hours after the challenge was observed (96.69 ± 22.01; 96.23 ± 22.23). A measurement of PEF remained stable (454.33 ± 60.86 vs 461.62 ± 65.95) (table I).

Choosing SPT wheal size in NPT positive and negative patients (median SPT wheal size: 9 mm vs 5 mm; challenge positive vs negative patients), the optimal skin prick wheal size cut off for Sk was determined using ROC curves, constructed by plotting sensitivity vs specificity at various skin prick wheal size diameters for Sk provocation positive and negative patients (figure 1). SkSPT at threshold of > 7.5 mm enabled the identification of Sk provocation positivity with sensitivity of 73.6% and specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; p < 0.0001) (table II, figure 1).

Discussion

Although advanced diagnostic tools in allergy might improve the selection of patients for AIT (17), SPT is highly specific (79-86%) and sensitive (9) (85-87%), and remains the technique of choice in allergy practice for identification of causative allergens in patients with allergic rhinitis. The reliability of SPT depends on the skill of the tester, the test instrument (14), potency and stability of test reagents, skin colour and patient’s age, as well as

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**Figure 1 - ROC analysis for Salsola kali positive and negative SkNPT.**

![ROC analysis for Salsola challenge (+) and (-) patients](image)

**Table II - Sensitivity and specificity at different cut off of SPT to Salsola kali in regard of SkNPT positivity.**

<table>
<thead>
<tr>
<th>Cut off of Salsola SPT</th>
<th>Sensitivity (%)</th>
<th>95% confidence interval (CI)</th>
<th>Specificity (%)</th>
<th>95% confidence interval (CI)</th>
<th>Likelihood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 6.500</td>
<td>86.40</td>
<td>79.12% to 91.87%</td>
<td>84.62</td>
<td>65.13% to 95.64%</td>
<td>&gt; 6.500</td>
</tr>
<tr>
<td>&gt; 7.500</td>
<td>73.60</td>
<td>64.97% to 81.08%</td>
<td>100.0</td>
<td>86.77% to 100.0%</td>
<td>&gt; 7.500</td>
</tr>
<tr>
<td>&gt; 8.500</td>
<td>50.40</td>
<td>41.32% to 59.46%</td>
<td>100.0</td>
<td>86.77% to 100.0%</td>
<td>&gt; 8.500</td>
</tr>
<tr>
<td>&gt; 9.500</td>
<td>37.60</td>
<td>29.10% to 46.70%</td>
<td>100.0</td>
<td>86.77% to 100.0%</td>
<td>&gt; 9.500</td>
</tr>
<tr>
<td>&gt; 10.50</td>
<td>16.80</td>
<td>10.71% to 24.53%</td>
<td>100.0</td>
<td>86.77% to 100.0%</td>
<td>&gt; 10.50</td>
</tr>
<tr>
<td>&gt; 11.50</td>
<td>10.40</td>
<td>5.655% to 17.13%</td>
<td>100.0</td>
<td>86.77% to 100.0%</td>
<td>&gt; 11.50</td>
</tr>
</tbody>
</table>
(6). Those factors, besides the lack of SsIgE cut offs based on the SPT, might influence the interpretation of SPT (18). A positive immediate skin reaction at the threshold of a 3 mm wheal of the longest (19) or mean diameter (6) is a widely accepted criterion. However, 3 mm wheal threshold might lead to the overestimation of allergic disease and increase the risk of inadequate AIT (20). To improve clinical interpretation of SPT results in terms of its clinical relevance, Haahatala et al. (21) calculated quantitative decision points for 18 inhalant allergens with the wheel size in mm and found that the risk of allergic symptoms to particular allergen increased significantly with larger wheal sizes for 17 of the 18 allergens tested (the 80% PPV varied from 3 to 10 mm depending on the allergen). Similar observation was documented in our study. The mean ± SD of Sk wheal longest diameter size was 8.24 mm ± 2.79 mm (table I). However, 15% of our SPT positive patients on threshold of 3 mm wheal did not react to nasal provocation. Furthermore, we observed that all Sk provocation negative patients had a significantly lower wheel diameter in comparison with those who reacted positively (4.88 ± 1.21 vs 8.94 ± 2.5; p < 0.0001) (table II). This observation supports results obtained by others (22), that larger skin reactions predict higher likelihood of positive nasal response and better correlate with clinical allergen reactivity with inhalant allergens, as well. Zarei group (12), using cat NPT, documented that a 3 mm skin prick wheal will overestimate the presence of cat allergy. They found that a 6 mm wheal size appears to distinguish those individuals who are cat allergic from those who are not. The authors concluded that instead of taking skin prick wheal cut offs of 3 mm as standard criterion, the prick wheel size cut off for each allergen should be determined. Similar results are documented by others (23). These results are in concordance with ours from the present study, as well as our other study (24) done with a cat allergen. We found, similar to Zarei group (12), that positive cat NPT detected true cat allergy in an environment with a low cat ownership, that was predicted by a cat SPT wheal size > 6.5 mm with a sensitivity of 71.11% and a specificity of 100%. Nasal provocation is more specific, accurate (13) and safe (25) test that is considered as the best diagnostic “gold standard” (16), if culprit allergen is elusive. Accuracy of NPT in this study was supported with results obtained by objective measurements of nasal patency using PNIF, and by safety profile, showing no significant changes in PEF rate during procedure for all patients. NPT is a valuable method in determining cut off level of SPT wheal, whenever discordance between clinical history and SPT and/or SsIgE is present. In the absence of a positive NPT, positive SPT results might be related to the presence of cross-reactivity between weed pollen species (10, 20, 26). Furthermore, the amount of pollen each subject is exposed to, in real life, depends on several uncontrollable factors like climate, lifestyle and the actual pollen load in the air (27).

We used receiver operating characteristic (ROC) curves to determine optimal cut off values by plotting sensitivity vs specificity at various skin prick wheal diameters for Sk, challenge positive and negative patients. Results are shown on table II and figure 1. We observed that SkSPT at the threshold of > 7.5 mm enabled the identification of Sk provocation positivity with a sensitivity of 73.6% and a specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; p < 0.0001) (table II). Therefore, patients eligible for SkAIT whose SPT wheal is less than 7.5 mm should be taken into consideration to carry out nasal provocation to verify a clinically relevant allergen. Similar suggestions are given by others (28).

As a limitation of the current study, a relatively small number of patients were included. Due to missing data in majority of included patients, the correlation of SsIgE with SPT wheal cut off was not evaluated. In addition, it has been previously shown that patient’s age might influence on SPT cut offs for different inhalant allergens (11): since our group of patients was relatively homogenous in regard to age, we have not focused on this issue. In conclusion, a SPT wheel size ≥ 7.5 mm for Sk might be considered as an appropriate wheel size in confirming Sk allergy in adult patients with moderate to severe SAR. SkNPT might be recommended if SkSPT wheel size is < 7.5 mm. Selection of the proper SPT wheal cut off value rather than using a uniform value might be important in the accurate treatment with AIT. More studies with higher number of patients with moderate to severe SAR sensitized to other allergens typical for desert climate, are necessary. Furthermore, similar evaluation of cases with a mild SAR, in comparison to more severe form of allergic rhinitis, would be interesting for potential AIT, which is still the only treatment modality capable of preventing further progression of allergic disease.

Conclusions

SPT wheal size of 3 mm might overestimate the presence of real allergy to Sk in a desert environment. A SPT wheal size > 7.5 mm for Sk appears to distinguish those individuals who develop disease from those who does not. Physicians should select the proper SPT wheal size value as an appropriate criterion according to the allergen rather than using a uniform cut off value in patients eligible for AIT.

Ethics

All patients were informed about the risk and outcomes of the procedure and provided informed consent.

Conflict of interests

The authors declare that they have no conflict of interests.
References

A preliminary study to investigate effectiveness of a mixed extract of *Dermatophagoides* sp. house dust mites and *Alternaria* sp. mold

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Key words
Real-world evidence; subcutaneous immunotherapy; house dust mites; *Dermatophagoides; Alternaria; quality of life.*

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Summary
Background and objective. Although the administration of single-allergen extracts is recommended, there are polysensitized patients who require a different strategy. This study evaluates the effectiveness of an extract containing a mixture of house dust mites (HDM) and mold allergens in polysensitized patients with asthma and/or rhinitis. Methods. Using validated questionnaires, we assessed asthma and rhinitis control and quality of life (QOL) of patients that received a combined immunotherapy of HDM and mold in routine clinical practice. Results. 39 polysensitized patients with asthma and/or rhinitis were included. After 6 months of follow up, asthma control increased significantly from baseline and was maintained at 12 months. However, QOL of asthma patients did not change significantly from baseline to month 6 or 12, but at month 12, 57.9% of them improved their score and 5.3% maintained the same. On the other hand, QOL of 76.9% patients with rhinitis improved significantly at both 6 and 12 months. Conclusions. In this preliminary study, the administration of immunotherapy based on the combination of allergens from HDM and mold, besides being effective, also allows an increase in the quality of life of patients with asthma and/or rhinitis.

Impact statement
A mixed extract with house dust mites and molds shows effectiveness and improves quality of life of patients.

Introduction
Allergen immunotherapy (AIT) is the only procedure currently available that can modify respiratory allergy. Its objective is to reduce allergic symptoms and drug intake (1, 2). This type of treatment can be administered subcutaneously through injections over a period of years, or sublingually through the daily or almost daily administration of allergen extracts.

The European Medicines Agency (EMA) recommends the development and administration of AIT using a single allergen or mixtures with as few allergens as possible, trying to avoid mixtures of seasonal and perennial allergens or allergens with proteolytic activity (3). However, many patients with respiratory allergy are sensitized to multiple allergens. Although AIT has been shown to be effective in polysensitized patients, its management has not yet been standardized (4). The main criteria in these patients are based on identifying the most clinically relevant allergens and administering immunotherapy with the allergen causing the most intense and bothersome symptoms. Another alternative is to administer two vaccines consecutively when two of the major allergens cause a significant deterioration in the patient’s quality of life (4).

A survey of current clinical practice found that 58% of polysensitized patients were treated with AIT based on a single allergen extract, while the remaining 42% were treated with AIT based
on a combination of allergens, either in the same vial or in parallel (5). These results highlight the need for further studies with AIT in polysensitized patients, especially under clinical practice conditions. Such studies will provide results under real-world conditions, as the controlled trials do not represent the actual patient population observed in clinical practice (6).

This is the first preliminary study conducted under routine clinical practice conditions in which the effectiveness of an extract containing a mixture of *Dermatophagoides* sp. mite allergens and *Alternaria* sp. mold is evaluated in patients with asthma and/or rhinitis that are polysensitized to HDM and mold.

**Methods**

**Study design**

Observational, retrospective, and interventional study conducted under routine clinical practice conditions in the Allergology Clinic of La Plana Hospital in Vila-Real (Castellón, Spain), in which the quality of life of patients sensitized to HDM and mold receiving immunotherapy with a mixture of *Dermatophagoides* sp. mites and *Alternaria* sp. mold allergens was evaluated. All patients gave verbal informed consent. The study was approved and validated by the Ethics Committee of the hospital, and conducted in accordance with the ethical standards established in the Declaration of Helsinki of 1946.

**Study population**

From all the patients treated with specific AIT who attended the specialized Allergology Clinic, we selected those with asthma and/or rhinitis, with clinically relevant sensitization to the HDM *Dermatophagoides pteronyssinus* and *D. farinae*, and to the mold *Alternaria alternata*, and who had also been treated with immunotherapy based on a mixture of allergens of these species (Allergovac Rapid®, Roxall, Zamundio, Spain). These patients met the usual criteria for receiving immunotherapy: 1) specific IgE antibodies to relevant allergens by skin prick test; 2) symptoms clearly related to exposure to the allergen; and 3) persistence of symptoms after adequate implementation of prophylactic environmental control measures.

**Study treatment**

Allergovac Rapid® vaccine consists of a sterile suspension of allergenic extracts absorbed in aluminium hydroxide in 0.5% phenol salt solution and 0.3% human serum albumin. Some of the main allergens are as follows: Der p 1 0.88 µg/ml, Der p 2 1.38 µg/ml, Der f 1 0.68 µg/ml, Der f 2 0.89 µg/ml and Alt a 1 0.07 µg/ml. The vaccine is presented in two vials with increasing concentrations that are administered subcutaneously in alternate arms every week for the first 4 weeks, and then every 4 weeks.

After completion of all doses of Allergovac Rapid® vaccine, maintenance was performed with a sterile suspension of an extract of *D. pteronyssinus*, *D. farinae*, and *A. alternata* (Allergovac Depot®, Roxall, Zamundio, Spain). This vaccine contains the same concentrations of allergens as the starting vaccine and is administered every 4 weeks.

**Outcome measures**

Several questionnaires were completed at the time of the vaccine administration, at 6 and 12 months later: Patients with asthma used the Asthma Control Test (ACT) and the Asthma Quality of Life Questionnaire (AQLQ), and patients with rhinitis completed the ESPRINT-15 questionnaire, which evaluated the control of the disease and the quality of life of patients. All questionnaires were validated for the Spanish population (7-9). The ACT questionnaire is used to assess the degree of asthma control in a simpler way than other questionnaires, such as the Asthma Control Diary (ACD) (10) or the Asthma Control Questionnaire (ACQ) (11). In addition, this questionnaire can provide the physician with information on the patients’ perception of the degree of control of their disease. The main advantage of ACT is that allows to assess asthma control without pulmonary function values. It consists of 5 items covering 5 dimensions: difficulty in breathing, use of emergency medication, impact of asthma on daily activities, nighttime awakening, and perception of asthma control. Each item is scored from 1 to 5, so that the overall score ranges from 5 (worst possible asthma control) to 25 (optimal control) (9).

The AQLQ test measures the quality of life of patients with asthma by evaluating both the physical and emotional impact of the disease. It consists of 32 items grouped into 5 dimensions: symptoms (11 items), emotional function (5 items), environmental exposure (4 items), and activity limitation (12 items, of which 5 refer to daily activities that the patient can choose from a list of 26 possibilities). The responses for each item range from 1 (severely affected) to 7 (unaffected) (7).

The ESPRINT-15 questionnaire consists of 15 items distributed in 5 dimensions: symptoms (5 items), daily activities (3 items), sleep (3 items), psychological impact (3 items), and general health (1 item). The score obtained, either globally or for each dimension, ranges from 0 (no impact on quality of life) to 6 points (maximum impact on quality of life) (8).

**Statistical analysis**

The primary endpoint was the absolute and relative change in the mean ACT, AQLQ, and ESPRINT-15 scores from the time of vaccine administration to 6 and 12 months. The Wilcoxon non-parametric test was used to evaluate the ACT scale. The sample size was estimated considering an alpha error of 0.05 and a beta error of 0.20 and therefore a power of 80%.
Assuming a 50% reduction in the mean score of the questionnaires, it was estimated that 24 patients were needed. The qualitative variables will be analyzed with their frequency distribution and their association by means of the Chi-square test. Quantitative variables were expressed as means and standard deviation (SD) in the case of following a Gaussian curve distribution, or as medians and interquartile ranges in the case of asymmetry. The 95% confidence intervals (95% CI) of the effects obtained were calculated when necessary and their statistical significance by Student’s t test for paired data. An ANCOVA analysis was performed to adjust for confounding factors. The analysis of the data was done using the program STATA ver. 12.0.

Results

Patient characteristics

A total of 39 patients with a mean age of 21.19 years were included; 48.7% suffered from rhinitis and the other 48.7% from rhinitis and asthma. Only 2.6% suffered only from asthma. Sixty-four per cent presented co-sensitization (table I). Some of these sensitizations were to olive tree pollen (3 cases, 7.7%), grasses (2 cases, 5.1%), or a combination of olive tree and Parietaria sp. pollen (2 cases, 5.1%). Each of the other patients showed different sensitization to a combination of various antigens.

Asthma Control Test results

There was a statistically significant improvement in asthma control according to the ACT from the administration of the vaccine to 6 and 12 months (p = 0.007 and p = 0.042, respectively) (table II). At baseline, the score was 18.24 (partially controlled asthma), and at 6 and 12 months, 21.39 and 22.0 points were reached respectively (well controlled asthma). Of the 18 patients assessed at 6 months, 12 patients (66.7%) improved their score, 3 (16.7%) maintained the same score, and 3 (16.7%) worsened. Of the 21 patients assessed at 12 months, 11 (55.0%) improved, 3 (15.0%) maintained, and 6 (30.0%) got worse.

Asthma Quality of Life Questionnaire results

According to the AQLQ questionnaire, asthma-related quality of life did not vary significantly between the start of treatment and 6 or 12 months after, both globally and in each of the dimensions assessed: chest heaviness, emotions, concerns, and social (table III). According to the global score, of the 17 patients evaluated at 6 months, 11 (64.7%) improved, 1 (5.9%) maintained the same score, and 5 (29.4%) got worse. Of the 19 patients assessed at 12 months, 11 (57.9%) improved, 1 (5.3%) maintained, and 7 (36.8%) got worse.

Rhinitis control and quality of life for patients with rhinitis

According to the ESPRINT-15 questionnaire, the 39 patients evaluated described a significant improvement in quality of life at both 6 and 12 months after starting the administration of the vaccine (table IV). This improvement was observed both in the overall score and in each of the 5 dimensions evaluated. Of the 35 patients assessed at 6 months, 27 (77.1%) improved their score, 1 (2.9%) obtained the same score as at baseline, and 7 (20.0%) worsened. Of the 39 patients assessed at 12 months, 30 (76.9%) improved their score, 3 (7.7%) maintained the same score, and 6 (15.4%) got worse. According to item 15, referring to how patients describe their health considering only rhinitis, 79.5% said it was excellent, very good, or good at the beginning of treatment, 82.1% at 6 months, and 89.7% at 12 months. Both at the beginning and at 6 months, only one patient con-
House dust mites and molds could be mixed in allergen immunotherapy extracts

Table III - Asthma Quality of Life questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>Basal (N = 20)</th>
<th>6 months (N = 17)</th>
<th>12 months (N = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>2.12 (0.38)</td>
<td>1.52 (0.39)</td>
<td>1.43 (0.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.179</td>
<td>p = 0.157</td>
</tr>
<tr>
<td><strong>Chest heaviness</strong></td>
<td>2.00 (0.38)</td>
<td>1.62 (0.45)</td>
<td>1.67 (0.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.379</td>
<td>p = 0.509</td>
</tr>
<tr>
<td><strong>Emotions</strong></td>
<td>2.30 (0.53)</td>
<td>1.79 (0.46)</td>
<td>1.50 (0.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.293</td>
<td>p = 0.255</td>
</tr>
<tr>
<td><strong>Concerns</strong></td>
<td>1.71 (0.38)</td>
<td>1.34 (0.39)</td>
<td>1.29 (0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.280</td>
<td>p = 0.517</td>
</tr>
<tr>
<td><strong>Social</strong></td>
<td>1.70 (0.45)</td>
<td>1.28 (0.46)</td>
<td>1.26 (0.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.138</td>
<td>p = 0.527</td>
</tr>
</tbody>
</table>

AQLQ: Asthma Quality of Life Questionnaire; SD: standard deviation.

considered his health to be poor. However, at 12 months no patient considered their health to be poor.

**Discussion**

This is the first study that evaluates data collected on the use of immunotherapy based on a combination of HDM and mold allergens in patients sensitized with asthma and/or rhinitis in routine clinical practice. The results gathered in this study have shown that this allergen-mixed vaccine significantly improves asthma and rhinitis control and the quality of life of patients. Until now, it was assumed that this type of mixture was not functional because the enzymatic activity of some allergens compromised the structural integrity of others (12-17). However, a study by Grier et al. showed that immunotherapy based on a combination of allergens from different species is possible (18). Their hypothesis was based on the ability of glycerin to inhibit the proteolytic activity of allergens in the extracts. Using variable concentrations of glycerin, mixtures were prepared with allergens from HDM, molds, plants, and animals from different sources. The study showed that some of the mixtures considered as unstable, had a considerable biochemical compatibility, which opened the possibility of developing new formulations, doses, and treatments for patients (18). In fact, in our study we were able to verify that a mixture of allergens from mites and mold is effective.

Studies conducted so far have evaluated the efficacy of subcutaneous immunotherapy based on mite extract and that based on fungal extract separately (19-22). One of these studies assessed the subcutaneous administration of different doses of long-acting D. pteronyssinus extracts to patients with allergic rhinoconjunctivitis (19). Compared with placebo, subcutaneous administration of an extract of these mites reduced sensitization to allergens, increasing IgG levels and decreasing IgE levels. In addition, this effect increased with the dose of the extract (19). Another study assessed the safety and tolerability of subcutaneous administration of a long-acting D. pteronyssinus extract to patients with allergic rhinoconjunctivitis with or without asthma who are sensitized to this mite (20). The results of the study showed a good safety and tolerability profile of the extract, providing a significant increase in IgG and IgG4 compared with placebo (20).

Other studies did the same with extracts from mold. In one of them, the effect of an extract of A. alternata was analyzed in patients with rhinitis and/or bronchial asthma sensitized to this mold (21). A decrease in conjunctival reactivity was observed in a provocation test, although there was no change in the skin reactivity of the prick test. A decrease in IgE levels and an increase in IgG, IgG1, and IgG4 levels were also observed (21). Another study evaluated the safety and efficacy profile of A. alternata extract in patients with rhinitis and/or bronchial asthma who are sensitized to the mold (22). Six months after administration of the extract, a significant improvement in respiratory symptoms compared to placebo was observed, decreasing both the severity of asthma and rhinitis (22).

In the latter study, the evaluation of the quality of life of patients using the AQLQ questionnaire is also relevant (22). Although treatment improved the quality of life of both asthma and rhinitis patients compared to placebo, the difference was not statistically significant. A significant improvement was only observed in the symptom dimension in patients with asthma, and in the
emotional state dimension in patients with rhinitis (22). In our study, we observed an improvement in all dimensions of the questionnaire, but none were statistically significant with respect to baseline, probably due to short duration of AIT at that moment. According to the study that established reference values for the ESRINT-15 questionnaire based on the severity of allergic rhinitis, the mean scores for patients with moderate to severe persistent rhinitis were 2.6 (SD 1.2) in men and 2.7 (SD 1.3) in women (23). In our study, the baseline score was 2.38 (SD 0.23), which would classify patients as having moderate to severe persistent rhinitis. The administration of the combination vaccine allowed a statistically significant reduction in this value to 1.63 (SD 0.24) 6 months later (\( p = 0.001 \)) and to 1.45 (SD 0.18) 12 months after starting treatment (\( p < 0.001 \)).

The main limitation of this study is the common one in retrospective studies conducted in clinical practice, especially regarding the establishment of a control group. For this reason, the results do not necessarily have to be universally valid or be extrapolated to other groups of patients, although they do open the way to further studies to corroborate and extend the results obtained in this one.

Conclusions

The results of our preliminary study show that the administration of specific immunotherapy based on the combination of allergens from the mites *D. pteronyssinus* and *D. farinae*, and the mold *A. alternata*, seems to be effective and possibly able to increase the quality of life of patients with asthma and/or rhinitis. This outcome needs to be confirmed by randomized placebo-controlled trials.

Fundings

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Conflict of interests

The authors declare that they have no conflict of interests.

References

To the Editor,

as severe allergic reactions to the first authorized COVID-19 vaccine, BNT162b2 developed by Pfizer and BioNTech, were reported (1), concern arose among clinicians and in the general population – especially among allergic patients – with the possible consequence of contributing to vaccination hesitancy. The lipid nanoparticle (LNP) transporting the mRNA was immediately considered as the possible target of the allergic response, as it hides several potential culprits (figure 1).

Among these, it has been noted that lipid ALC-0159 contains PEG-2000, that belongs to the polyethylene glycols family, which are known to be capable of inducing hypersensitivity reactions (1). These are polymers with similar structures but different molecular weight, which are widely used in pharmaceuticals, cosmetics or foods thanks to their solubility and stability (2). Despite their ubiquity, however, immediate hypersensitivity reactions are rare: seventy-four cases were reported in the last 40 years (2). Immunoglobulin E (IgE)-mediated hypersensitivity is one of the suspected mechanisms, and sensitization could have taken place during repeated exposure of offended skin/mucosa to cross-reactive PEGs (2, 3). The PEGs’ multivalent structure favors cross-linking of FcεRI, thus inducing mast cell degranulation. Complement pathway activation by PEG has been proposed (5); although PEGs prevent opsonization, they could induce IgM and IgG production (6). Nevertheless is not a single PEG but the presence of highly repetitive domains in LNP resembling a pathogen surface that can induce the so-called complement activation-related pseudoallergy (CARPA) (4). The same mechanism can be advocated when engineered nanomaterials, e.g., liposome and micellar drugs, bind on their surface proteins creating a “biocorona”. And even more other pathways leading to mast-cells degranulation have been identified (5) or postulated, including direct mast cell activation and degranulation (6).

PEG could therefore be the culprit of hypersensitivity reactions to BNT162b2 vaccine, but it should be noted that other vaccines that contain PEG cross-reactive excipients (e.g., polysorbate 80) induce hypersensitivity reactions with significantly lower incidence than those reported for mRNA COVID vaccines.
Apart from PEG, another component of the LNP, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), should also be considered a potential culprit as it contains a quaternary ammonium (QA) ion (Figure 2) (7).

Steroidal ammonium neuromuscular blocking agents (S-NMBA), i.e., rocuronium and similar, also contain this ion, and it is known that IgE-mediated hypersensitivity reactions to QA (but also other possible mechanisms, e.g., activation of Mas-related G-protein-coupled receptor member X2 (MRGPRX2)) are implicated in anaphylaxis that can occur even at first exposure with these drugs, thus resembling the aforementioned cases associated to Covid-19 vaccine (8). The origin of such sensitization against QA was identified in previous exposure to QA-containing compounds, such as cosmetics and disinfectants, through damaged skin/mucosa (9). Whether the BNT162b2 vaccine could exploit specific IgE to QA needs to be better evaluated.

No hypersensitivity reactions to the third component of the LNP, ALC-0315, have been reported to date to the best of our knowledge.

Apart from lipids, even the mRNA has immunogenic properties since it is capable of inducing an inflammatory response by itself. Toll Like Receptors (TLRs) and other Pattern Recognition Receptors (PRRs), such as RIG-I, can detect mRNA and activate...
the inflammation cascade (6, 10, 11); however, it is not known if this kind of mechanisms triggered by mRNA can lead to mast cell degranulation (12).

In conclusion, the culprit involved in the cases of immediate reactions to mRNA BNT162b2 vaccine seems to be concentrated mainly in the LNP constituting the envelope. Further research on this topic is crucial, as a better knowledge of the mechanism underlying adverse reaction to BNT162b2 could allow a better risk stratification and patient selection, thus increasing safety for people undergoing vaccination and, in turn, reducing vaccination hesitancy.

**Contributors**

All the authors have contributed to conceptualization, writing, revision of this letter. Anna Radice and Filippo Fassio created the figures.

**Conflict of interests**

The authors declare that they have no conflict of interests.

**References**

Covid-19: less bronchial airways, more lung alveolar space and blood ways

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Key words
Asthma; Covid-19; SARS-CoV-2; thromboembolism; endothelial dysfunction.

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To the Editor,

we report our experience in Brescia during the pandemic of Covid-19 by focusing on asthmatic patients affected by this infection, because we believe it is an interesting aspect to investigate. In late December 2019 many Chinese patients were hospitalized with an initial diagnosis of pneumonia of unknown aetiology. Subsequently, in the city of Wuhan, a new coronavirus was identified as responsible: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the related disease (COVID-19), named by WHO on February 11, 2020, has then developed into a global pandemic. Patients affected by COVID-19 suffer with fever, upper respiratory airways symptoms and pneumonia with the risk of respiratory failure and death; others symptoms are described too like ageusia, anosmia and gastrointestinal symptoms. In a recent article, by Richardson et al, the characteristics, comorbidities and outcomes of 5700 patients hospitalized with COVID-19 are analysed (1). Analysing the data reported by the authors on comorbidities it is interesting to note that there is no increase in hospitalizations of asthmatic patients: the reported percentage (9%) in the table is in line with the annual American one (2). Considering the COVID-19 characteristics, it should be expected to observe an increase of asthma exacerbations and hospitalizations, a typical and frequent consequences of viral respiratory infections, in patients with asthma comorbidity as happened in the pandemic of Swine Influenza A (H1N1) and in the Middle East Respiratory Syndrome (MERS). Instead, as in the United States even in our hospital, the percentage of asthmatic patients was in line with the Brescia (Lombardia Region, Northern of Italy) prevalence typical of this season (3). In the months of the pandemic explosion, especially from January to March 2020, our hospital in Brescia did not show an increase in emergency room admission of asthmatic patients if we compare these data with those relating to the same period of the previous year (January to March 2019). There has been no increase of these cases: 199 patients in the year 2020 vs 218 in the year 2019 (table I).
Furthermore, according to these data, the prevalence of asthma in COVID-19 positive patients admitted to our hospital was lower than in the general population (20 patients out of a total of 1043 positive hospitalised patients (1.92%), F/M = 12/8; age range: 41-77 years; mean age: 61 years). The asthma prevalence in Italy is 5-6% in the general population. All this astonish considering that normally there is a close association between asthma and viral respiratory infections, usually with an increased frequency and severity of lower respiratory tract infection in asthmatic patients and that asthma predisposes to airway dysfunction due to chronic inflammation (4).

These data are even more significant if we consider that the pandemic coincided with the seasonal increase in pollen counts in the Northern of Italy; despite this there were no asthmatic exacerbations in COVID-19 positive patients. Therefore, it appears that asthma is not a risk factor in the development of COVID-19 and that SARS-Cov-2 does not aggravate asthma.

A possible explanation is that therapies used by asthmatic patients can reduce the risk of infection or developing symptoms: inhaled steroids, alone or in combination with bronchodilators, could promote the suppression of viral replication and of the production of cytokines responsible for the inflammatory storm. 15 of our 20 patients were being treated with Long-acting beta-agonists (LABAs) in association with Inhaled corticosteroids (ICSs).

Another interesting aspect is that asthma could protect against COVID-19, perhaps through a different pattern of immune response elicited by the chronic disease itself. In particular a different immune response, in patients with allergic asthma, could lead to a higher production of natural antibodies than non-allergic one (5).

Is therefore COVID-19 only an exclusively airway disease? It is probably a more complex entity selectively affecting the alveolar space and the blood vessels (6).

To confirm this hypothesis there are several studies in the literature demonstrating the important role of thromboembolism (7). SARS-CoV-2 appears to have direct and indirect effects to thrombotic events, both in the venous and arterial circulations, so much so that haemostatic alterations, including disseminated intravascular coagulation (DIC), have been reported in patients with COVID-19. The authors claim that endothelial dysfunction, platelet activation, stasis and excessive inflammation may be the major factors implicated in the pathogenesis of thromboembolism (7).

Furthermore, by checking the accesses for thromboembolism at our hospital in the first three months of the year 2020 and comparing them with the same ones in 2019, we noticed an increase in cases: 32 patients (of which 25 patients positive for SARS-CoV2 on nasopharyngeal swab) compared to 14. Statistical analysis of data confirmed that this increase was very significant: the standardized value of the sample is 9.36, thus the associated p-value is smaller than $10^{-16}$ (table I).

The thromboembolic phenomenon is responsible for the high mortality rate of COVID-19 showing that, probably, this is the major complication due to SARS-CoV-2 infection. Therefore, it is possible to hypothesize that COVID-19 probably represents a more complex pathology than an airway infection only: it involves less airways and much more alveolar spaces and blood vessels.

**Conflict of interests**

The authors declare that they have no conflict of interests.

**References**


**Table I** - Patients admitted to emergency room at our hospital in the months January-March 2019 compared to 2020, stratified by asthma and thromboembolic disease.

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>Thromboembolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>79</td>
<td>2019</td>
</tr>
<tr>
<td>February</td>
<td>87</td>
<td>2020</td>
</tr>
<tr>
<td>March</td>
<td>52</td>
<td>2019</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2020</td>
</tr>
</tbody>
</table>

* Value of the sample is 9.36, thus the associated p-value is smaller than $10^{-16}$. 
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