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35 years of autologous serum skin test in chronic spontaneous urticaria: what we know and what we do not know

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Key words

Chronic spontaneous urticaria; autologous serum skin test; autoreactivity; autoimmunity; histamine; histamine-releasing factors.

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Summary

The autologous serum skin test (ASST) has been used in patients with chronic spontaneous urticaria (CSU) as a means to detect an autoreactivity state for thirty-five years now. Nonetheless, several aspects of this old diagnostic test are still insufficiently defined. Particularly, the nature of the factor(s) responsible for the appearance of the wheal-and-flare skin reaction is still poorly characterized. This article will review our current knowledge about the clinical significance of the ASST and the factors possibly associated with the occurrence of the skin reaction following the intradermal administration of autologous serum that are known so far.

Impact statement

35 years after its introduction in the clinical practice, the autologous serum skin test still shows some unclear aspects that are addressed by the present review.

Introduction

Chronic spontaneous urticaria (CSU) is a quite common and frequently very disturbing disease characterized by the recurrent eruption of itchy wheals, often associated with angioedema for more than 6 weeks. The pathogenesis of CSU has remained obscure for decades until Grattan and co-workers, more than 30 years ago (1), found that a significant proportion of CSU patients develop a wheal-and-flare reaction in the site of the intradermal injection of a small amount of autologous serum (autologous serum skin test, ASST). Grattan's work actually resumed and developed the observations of Malmros, who evaluated the effects of intradermal injection of autologous serum in 956 patients with different disorders and found a wheal-and-flare reaction in 53 patients, 16 of whom with asthma and 6 with urticaria (2). The paper by Grattan and co-workers prompted the existence of circulating histamine-releasing factors in patients with CSU and has been the milestone of all subsequent studies that have led to our current understanding of the pathogenesis of this disease. We now know that the large majority of CSU patients have either an auto-allergic disease (type I, after Gell and Coombs), characterized by IgE directed against an array of auto-allergens (3), or an autoimmune disease (type IIb, after Gell and Coombs) characterized by functional IgG autoantibodies to IgE or to the high-affinity receptor (FcεRI) (4, 5). Recent studies found that the latter autoimmune process may be also IgM and/or IgA-mediated (6), and that the autoimmune and the auto-allergic pathogenic mechanisms co-exist in many patients (7) influencing to various extent their response to anti-IgE therapy. The ASST has been the silent witness in the background of all these advances throughout the decades, although its nature and clinical significance are far from being fully understood. The present article will review our knowledge about this "old" diagnostic method and will try to highlight the doubts that still surround its nature and clinical significance.

The ASST as a test for autoreactivity

About 10 years ago, two taskforces of the European Academy of Allergy and Clinical Immunology (EAACI), authored a couple of position papers, one dealing with the correct way to perform the ASST (8), and the other establishing criteria to define autoimmune urticaria (9). The task forces concluded that the ASST should be regarded as a test for autoreactivity rather than a specific test for autoimmune urticaria, as it shows only moderate specificity as a marker for functional autoantibodies against IgE or the high affinity IgE receptor (FcεRI) (8). Nonetheless its negative predictive value for autoimmune urticaria seemed excellent (8).
What does "autoreactivity" mean? A number of studies of ASST in CSU have been carried out in the past, showing large variability in positive skin testing, ranging between 4% and 76% (10). Although this may reflect differences in patients' selection and interpretation of positive results, the fact remains (and is generally accepted) that the ASST scores positive only in a variable proportion of chronic urticaria patients. In other terms, no "autoreactivity" can be detected in a variable proportion of patients with CSU.

The relationship between the ASST and serological tests for autoantibodies to IgE and/or FcεRI

The relationships between the ASST and functional autoantibodies against IgE or the high affinity IgE receptor were investigated in a study carried out 20 years ago on > 300 CSU patients (11). In that study, the ASST scored positive in 67% of patients whereas sera from only 16.5% of patients were able to induce histamine release from basophils of normal donors (BHRA, basophil histamine release assay), suggesting the presence of functionally active histamine releasing autoantibodies. Interestingly, all BHRA+ patients showed a positive ASST, but only 22% of ASST+ patients were also BHRA+. In BHRA+ patients, ultrafiltered serum fractions > 100 kDa fully retained their *in vitro* histamine-releasing ability, whereas serum fractions < 100 kDa were unable to induce any histamine release from donors' basophils. Notably, the proportion of patients whose sera were able to induce histamine release from basophils of normal donors is very close to that of patients that were identified as having an autoimmune chronic spontaneous urticaria (aiCSU) in a recent international study (12).
One very important (but rather overlooked) study about the lack of relationship between the ASST and functionally active, histamine-releasing autoantibodies was published by an Italian group in 2000 (13). In that study, heat-decomplemented/IgG-depleted sera elicited wheal-and-flare reactions on intradermal testing that were comparable with those observed with untreated sera. The authors concluded that skin reactivity to autologous serum was caused by unidentified non-Ig reactants. This observation was in line with earlier findings by Grattan and co-workers who observed that heat-decomplemented serum retained its ability to induce a wheal-and-flare reaction and that a low molecular weight histamine-releasing factor (HRF) could be detected in sera from CSU patients (14).
In summary, these studies show that all BHRA+ patients score positive on ASST, whereas most ASST+ patients do not show functionally active circulating histamine-releasing autoantibodies, suggesting that autoreactivity on autologous skin testing can be caused by serum factors other than autoantibodies.
The basophil activation test (BAT) and the ASST did not show significant correlation in some older studies (15, 16), but in a more recent, international study (12) ASST-positive patients included virtually all patients whose sera scored BAT-positive. In contrast, a much larger discrepancy between positive ASST and sera positive for IgG anti-FcεRl or IgG anti-IgE detected by ELISA was observed (12).

Effect of anticoagulants on autologous skin test

The first observations about the inhibitory effect of heparin on the autologous serum skin test were made by Fagiolo and coworkers in 1999 (17). They found that a positive skin test with autologous serum turned into negative if heparinized autologous plasma was injected intradermally, and if heparin was added to autologous serum. Further, adsorption of CSU sera with solid-phase heparin abrogated or strongly reduced the ability to induce cutaneous reactions. In contrast, interestingly, the intradermal injection of EDTA (ethylene diamine tetra acetic acid) - anticoagulated plasma did not modify the results of the ASST, and no change in the cutaneous response to allergens was associated with locally administered heparin in five atopic patients with no history of CSU. The authors concluded that heparin inhibits the cutaneous response to HRFs present in the sera of patients with CSU, possibly by a direct interference (17). These findings were confirmed two years later by another Italian group (11). In that study 205/306 (67%) CSU patients scored positive on ASST, whereas only 8/57 (14%) responded to intradermal injection of autologous heparinized plasma. Notably, all those 8 patients were very strong ASST reactors. As reported in Fagiolo's study (17), the addition of heparin to a commercial grass pollen extract did not change the wheal-and-flare response induced by skin prick test (SPT) in grass pollen allergic subjects. Further, *in vitro*, heparin dose-dependently inhibited histamine release induced by sera and plasma, and by basophil agonists such as anti-IgE, formyl-methionyl-leucyl-phenylalanine, and interleukin (IL)-3. The study concluded that heparin inhibits histamine release from both basophils (*in vitro*) and mast cells (*in vivo*), possibly acting directly at a cellular level (11).
A study published in 2006 (18) reported for the first time that the intradermal injection of autologous plasma anticoagulated with sodium citrate produced much more frequently a wheal and flare reaction than autologous serum in CSU patients (86%

vs 53%, respectively). This observation, along with the detection of increased levels of plasma prothrombin fragment 1+2 paralleling urticaria severity, led to conclude that CSU is associated with the generation of thrombin, and that APST (autologous plasma skin test) and ASST only partially depend on the presence of circulating antibodies to FcεRI or to IgE. This work represented the starting point of a number of subsequent studies dealing with the activation of the coagulation cascade in CSU and comparing APST and ASST that will not be considered here. In the same study (18), K_2EDTA was also tried as anticoagulant to test plasma skin reactivity, but it was found it caused nonspecific skin reactions that were directly related to its concentration both in patients and in controls.

Interestingly, it has been recently demonstrated that activated coagulation factors, such as factor Xa, factor IIa, and plasmin, can induce human skin mast-cell and basophil degranulation via the production of complement C5a which in turn binds to the C5a receptor and causes histamine release (19). This represents an additional IgE-independent mechanism of mast-cell and basophil activation which may be operating in CSU. Further, activated coagulation factors may activate mast cells via the so-called protease-activated receptors 1 and 2 (PAR-1 and PAR-2). Thrombin is able to activate PAR-1, while tissue factor+factor VIIa (FVIIa) and factor Va (FVa)+factor Xa (FXa) complexes act via PAR-2. This may well represent another IgE-independent pathomechanism playing a relevant role in CSU (20, 21).

Effect of antihistamines on autologous skin test

More recently, a study investigated the effect of H1-antihistamine treatment on the autologous plasma skin test in CSU patients, and found that 87% of them showed a large flare on APST while taking H1-antihistamines while the skin reaction to histamine 10 mg/ml was abolished or negligible (22). Little difference in the autologous plasma-induced flare was seen before and after the start of cetirizine therapy in 6 cases, whereas the drug exerted a marked effect on the histamine SPT as well as on the autologous plasma-induced wheal. The APST-induced flare was not associated with patients' response to H1-antihistamine. The study concluded that factors other than histamine are probably involved in the flare induced by APST in CU; such factors might play a pathogenic role particularly in patients not responding to standard H1-antihistamine treatments.

Are there other soluble histamine-releasing factors in sera from CSU patients?

In an *in vitro* study carried out using a mast cell line (HMC-1) missing the high affinity IgE receptor, most sera from CSU patients were able to promote the degranulation of mast cells, irrespective of a positive or negative autologous serum skin test (23). The study concluded that the combined degranulation and leakage assays used proved to be more sensitive than the ASST as a means to detect HRFs in patients with CSU. Subsequently, we found that both whole serum from CSU patients and serum fractionated at 100, 50, and 30 kDa, including fractions < 30 kDa, were able to activate LAD2 mast cells (carrying FcεRI receptors) significantly more than the corresponding fractions from normal control sera (24). These findings suggest that HRFs other than immunoglobulins may be involved in mast cell and basophil activation in CSU. In favor of this hypothesis also stands the observation that Mas-related G-protein coupled receptor-X2 (MRGPRX2), a protein that mediates IgE-independent activation of mast cells, basophils and eosinophils (25), is markedly upregulated in the skin of CSU patients (26). Some neuropeptides, such as substance P, and eosinophil-derived proteins, such as major basic protein and eosinophil peroxidase, can induce histamine release from human skin mast cells through MRGPRX2. It is conceivable that substance P as well as eosinophil-derived proteins may play a role in mast cell activation and contribute to CSU pathogenesis.

Other CSU-related conditions characterized by autoreactivity detectable by ASST

Patients showing a propensity to react (probably in a non-specific way) to several, chemically unrelated antibacterial drugs and termed as having a MDAS (multiple drug allergy syndrome) were found to show an extremely frequent skin reactivity (94%) upon intradermal injection of autologous serum (27). Interestingly, in the same study about one third of control patients with a history of hypersensitivity to a single antibacterial drug scored positive on ASST, whereas no normal control did. The study concluded that circulating histamine-releasing factors might play a role in drug-induced adverse reactions observed in these patients (27).

A similarly very high rate of positive ASST (91%) was detected in another study carried out in otherwise normal subjects with a history of hypersensitivity to multiple nonsteroidal anti-inflammatory drugs (NSAID) (28). These patients are currently defined as having a NIUA (NSAID-induced urticaria angioedema) (29). Interestingly, also in this case, ASST was positive in a significant proportion (36%) of patients with a history of single NSAID hypersensitivity (defined as having a SNIUA), whereas no normal control scored ASST-positive. One of these conditions, namely the NIUA seems quite correlated to CSU (30). The main features of ASST are summarized in **table I**.

Conclusions

There has been a rather limited interest in the ASST during the last few years, probably because attention was focused on the recent finding of autoreactive IgE supporting an "auto-allergic"

Table I *- Summary of the main features of the autologous serum and plasma skin test in CSU.*

Prevalence of positive ASST	Variable, generally about 50%
Addition of heparin to serum	Skin reactivity abolished
Skin test with heparinized plasma	Skin reactivity abolished
Skin test with K2EDTA anticoagulated plasma	Causes nonspecific positive reactions
Skin test with Na citrate anticoagulated plasma	Increases positive skin reactions in most studies
ASST with heat-decomplemented serum	No effect
ASST with IgG-depleted serum	No effect
Other CSU-related conditions characterized by ASST positivity	MDAS, NIUA

pathogenesis of the disease in a consistent proportion of CSU patients (2). Nonetheless, the nature and the clinical significance of this older diagnostic test remain an unsolved and fascinating problem. The evidence available so far suggests that skin reactivity to the intradermal injection autologous serum, albeit frequently associated with the presence of IgG autoantibodies directed against the high affinity IgE receptor or IgE, may also be due to HRFs other than antibodies. To our best knowledge, heparin is not able to bind immunoglobulins and inactivate them. In contrast, this might be the case with positively charged, low molecular weight histamine-releasing substances. In fact, heparin is a heavily negatively charged compound that might strongly bind and sequester similar substances. The demonstration of low molecular weight circulating HRFs in CSU patients and the immediate *in vivo* effect of heparin on ASST seems to support this view. In the light of the earlier findings of Grattan *et al*. on the occurrence of a low molecular weight serological mediator in CSU patients (14), and of our studies showing the presence of low molecular weight HRFs in sera from CSU patients (24) as well as their ability to activate human mast cells bypassing the high affinity IgE receptor (23), we believe reasonable to suppose their involvement both in the skin reaction to autologous serum and in CSU pathogenesis.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Clinical experience of a specialized urticaria outpatient clinic from a Portuguese UCARE

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Impact statement

Patients with chronic spontaneous urticaria, particularly severe cases, should be referred to urticaria specialists who have more diagnostic and treatment options at their disposal, offering a high degree of specialisation in terms of research and clinical patient care.

Summary

Background. *Chronic urticaria (CU) is a frequent disease, with a prevalence of at least 1%. It is characterized by pruritic wheals, angioedema or both for a period longer than 6 weeks.* ***Objective.*** *Identify the demographic, clinical, laboratory and therapeutic profile of patients treated in a Portuguese Urticaria Center of Reference and Excellence (UCARE) and compare it with international series.* ***Methods.*** *Retrospective analysis of database of patients observed in a specialized urticaria outpatient clinic, from January 2017 through September 2019, of a UCARE center in Portugal. Demographic and clinical features, laboratory findings and pharmacological treatment were obtained from the records. Descriptive analyses were performed for all variables. Chi square and fisher's exact tests were applied to analyze the independence of variables and the fit of distribution. P-value < 0.05 was considered significant.* ***Results.*** *During this period, 477 patients were observed, of whom 429 (90%) were diagnosed with chronic urticaria. Mean age (years) at the onset of symptoms was 43.7 (standard deviation (SD) 17.6, range 6-88) and at diagnosis 46.7 (SD 17.8, range 6-88) resulting in an average diagnostic delay of 3 years (range 0-25). Median follow-up period since first attendance in the specialized outpatient clinic was 1.7 years (interquartile range (IQR) 0.79, range 0.1-2.75). Concerning the whole group of CU patients, 347 (81%) had chronic spontaneous urticaria (CSU) – 79% female, 39 (9%) had isolated chronic inducible urticaria (CIndU) and 43 (10%) had CSU with CIndU. Autologous serum skin test (ASST) was done in 76 patients (positive in 24 (32%)) and basophil activation test (BAT) was done in 38 (positive in 13 (34%)). At the moment of study, 204 (48%) of CU patients were medicated with a second-generation H1-antihistamine (sgAH) daily (first-line therapy), 99 (23%) with sgAH up to four times the standard dose (second-line therapy) and 126 (29%) with omalizumab (third-line therapy). Additionally, 7 (2%) patients were completing a short course of systemic corticosteroids for management of disease exacerbation. Disease control was achieved in 316 of CSU patients (81%).* ***Conclusions****. Referral to a specialized urticaria outpatient clinic is important for a proper assessment of the disease and adequately symptom control.*

Introduction

Chronic urticaria is a frequent disease, estimated to affect at least 1% of the general population, and characterized by the appearance of pruritic wheals, angioedema or both that persist for more than 6 weeks (1, 2). A distinction is made between chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CIndU) (2, 3). In CSU symptoms are spontaneous and not associated with a specific trigger, as opposed to CIndU, in which urticarial symptoms only occur after exposure to definite external triggers, but not spontaneously (3). The triggers that lead to the urticarial signs and symptoms in CIndU patients are mainly physical or chemical stimuli. The former included pressure (in delayed pressure urticaria), radiation (in solar urticaria), friction (in symptomatic dermographism), temperature (in cold and heat urticaria), and vibration (in vibratory angioedema). Chemical triggers of CIndU reactions are water (in aquagenic urticaria), raised body temperature (in cholinergic urticaria), and other urticariogenic chemical compounds (in contact urticaria) (4, 5).

The second-generation H1-antihistamines (sgAH) are the first-line symptomatic treatment of patients with chronic urticaria. Up to 50% of the patients will not respond to licensed doses of sgAH. However, even at higher doses, there is a subgroup of patients refractory to the sgAH treatment and further treatment is frequently necessary (6). Omalizumab is an anti-IgE monoclonal antibody, approved for the treatment of CSU, that has radically changed the management of the patients without good response to sgAH, allowing to reach complete responses in a high percentage of patients (2). Omalizumab has also been shown to be effective and safe in the treatment of CIndU patients (3). It is, however, not approved for this indication, at least not in patients with sole CIndU.

Despite recent advances such as the global harmonization of chronic urticaria classification and nomenclature, novel diagnostic tools and instruments, and better treatment options, chronic urticaria can be a challenging condition for patients and their treating physicians. Urticaria Centers of Reference and Excellence (UCARE) have a strong network of urticaria specialists and, by promoting urticaria research, harmonize and improve urticaria management globally, helping to improve the management of this condition (7). Data regarding the demographic, clinical, laboratory and therapeutic characteristics of patients observed in UCAREs is valuable. It allows comparisons between published series in order to understand the similarities and differences between different centers and countries. Nevertheless, it has not been yet described in Portugal. We aimed to fill this knowledge gap by describing these features in a significant number of patients followed in a UCARE in Lisbon (Portugal) and compare it with international series.

Methods

The study was retrospective, based on the analysis of database of patients followed in the specialized urticaria outpatient clinic of a UCARE, Hospital Santa Maria, Centro Hospitalar Universitário de Lisboa Norte, in Portugal, from January 2017 to September 2019. This specialized Unit receives patients referred by other colleagues either working in hospitals or primary care units.

The diagnosis and etiology of chronic urticaria were defined by a detailed clinical history and classified using the national (8) and international European Academy of Allergology and Clinical Immunology (EAACI), the EU-funded network of excellence, the Global Allergy and Asthma European Network (GA²LEN), the European Dermatology Forum (EDF) and the World Allergy Organization (WAO) guidelines (2).

Other diagnosis, apart from chronic urticaria, as chronic pruritus, atopic dermatitis, prurigo strophulus, contact dermatitis, acute urticaria and hereditary angioedema were not included in the analysis.

Demographic and clinical characteristics were recorded: age and gender clinical data, urticaria subtypes (CSU, CIndU, CSU+CIndU), atopic comorbidities, time between the onset of symptoms and the diagnosis and follow-up period since first attendance in the specialized outpatient clinic.

Since we are a specialized urticaria outpatient clinic, the patients referenced usually have a more severe and/or long-lasting urticaria. Therefore, in patients with CSU, in addition to basic tests including differential blood count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), thyroid laboratory tests (TSH, free T4 (FT4) and thyroid autoantibodies (TAA)), namely thyroid peroxidase antibody (anti-TPO) and thyroglobulin antibody (anti-TG), were requested, as well as antinuclear antibodies (ANA), D-dimers (DD) and urea breath test to determine the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, especially in patients with long-standing and/or uncontrolled disease.

The patients with only CIndU were submitted to diagnostic tests adapted to each subtype, for identification of underlying causes or eliciting factors and for ruling out possible differential diagnoses.

The autologous serum skin test (ASST) is an *in vivo* test which assesses autoreactivity. Autoreactivity does not define autoimmune urticaria but may be an indication of mast cell activating autoantibodies in ASST positive CU patients (9). It was performed in patients with suspicion of autoimmune-related urticaria. The ASST and the basophil activation test (BAT) were done in patients with urticaria refractory to standardized dose anti-H1 treatment that tolerated discontinuation of these drugs for 5 days before testing and weren't doing any systemic corticosteroids for at least 2 weeks prior to the tests (9, 10). Therefore, in patients who were unable to suspend these drugs, it was not possible to perform the ASST.

The ASST was performed as recommended and described by the 2009 EAACI/GA²LEN task force consensus report on the ASST in urticaria. Wheal responses were measured at 30 min, and the ASST response was taken to be positive when the red serum induced wheal had a diameter at least 1.5 mm greater than the negative control (9). In BAT, briefly, blood from healthy donors was centri-

fuged and the buffy coat collected and resuspended in stimulation buffer containing IL-3. Donor basophils were added to each tube, and double staining was performed with anti-CCR3-PE and anti-CD63-FITC monoclonal antibodies (Bühlmann, Switzerland) and incubated at 37 °C for 15 minutes. Afterwards, the erythrocytes were lysed for 10 min at room temperature, and the samples were washed twice prior to analyzing them in a flow cytometer. Data were analyzed with FlowJo Tree Star software (Ashland, OR, USA). The test was considered positive when more than 5% of the total basophils were CD63 positive (10, 11). The disease activity and response to treatment was assessed by the Urticaria Activity Score 7 (UAS7), a validated diary-based Patient Report Outcome measure that assesses wheal number and itch severity scores, as described by the 2018 EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis, and management of urticaria (2). The UAS7 was calculated as the sum score of 7 days (minimum 0, maximum 42). Its values were assigned to five score ranges, reflecting urticaria-free to severe disease activity, as follows: UAS7 = 28-42 − severe activity CSU; UAS7 = 16-27 − moderate activity CSU; UAS7 = 7-15 – mild activity CSU; UAS7 = 1-6 – well-controlled CSU; UAS7 = 0 – urticaria-free (2, 12). It was used in all medical visits, but for the purposes of the study, it was considered the last value before the moment of analysis. Recommended treatment algorithm for urticaria includes 4 therapeutic steps: 1st line – sgAH standard dose; 2nd line – sgAH up to four times the standard dose; 3rd line – omalizumab; and 4th line – cyclosporine (2). It was also collected information about other types of medication used to manage disease exacerbations, namely oral corticosteroids.

Therapeutic modalities concerning the whole group of CU patients were characterized and were discriminated between CU subtypes (isolated CSU, CSU with CIndU and isolated CIndU). These apply only to treatment at the time of the analysis; no follow up or treatment modifications are described in this study.

Statistics

Descriptive analyses (frequency, percentage, mean, standard deviation, minimum and maximum values) were carried out for demographic and clinical variables. Chi-square test was used to analyze the fit of distribution and chi-square test/fisher's exact test to determine the statistical difference in qualitative variables. All statistical analyses were carried out using GraphPad Prism version 8.4.3 (GraphPad Software, Inc., CA, US). Significance was achieved with P-values < 0.05.

Ethical issues

The clinical part of the study as well as laboratory tests were carried out as part of the clinical routine of the urticaria specialized outpatient clinic. Patients gave an informed consent to the use of their clinical data in an anonymous form. All patients were treated according to ethical standards established in the Declaration of Helsinki.

Results

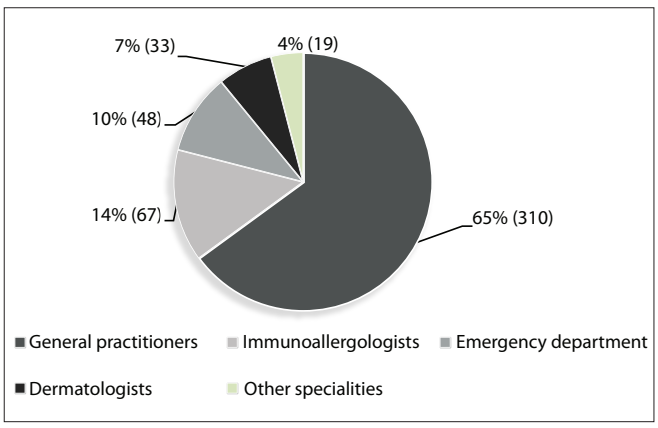
During the study period (January 2017 to September 2019), 477 patients were observed, of whom 310 (65%) were referred by the general practitioner, 67 (14%) by other immunoallergologists, 48 (10%) by the emergency department, 33 (7%) by dermatologists and 19 (4%) by other specialties, specifically internal medicine (8), rheumatology (n = 5), pediatrics (n = 3), hematology (n = 2) and nephrology (n = 1) (**figure 1**). Of the total of these patients, 429 (90%) were diagnosed with chronic urticaria (CU). Of the remaining 48 patients, 24 had other diagnosis (namely chronic pruritus (n = 13), atopic dermatitis (n = 8), prurigo strophulus (n = 2) and contact dermatitis (n = 1)), 19 had acute urticaria and 5 had hereditary angioedema. These patients were excluded for this analysis.

In the group of CU patients, mean age at the onset of symptoms was 43.7 years (standard deviation (SD) 17.6, range 6-88) and at diagnosis 46.7 years (SD 17.8, range 6-88), resulting in an average diagnostic delay of 3 years (range 0-25). Median follow-up period since first attendance in the specialized outpatient clinic was 1.7 years (interquartile range (IQR) 0.79, range 0.1-2.75). Before diagnosis, at our consultation or previously, 129 (30%) patients had symptoms for less than one year, 206 (48%) for 1-5 years and 94 (22%) for more than five years (**table I**).

More than half of these patients, 236 (55%), had history of atopic disease, with a predominance of allergic respiratory disease, namely 142 (60%) patients with allergic rhinitis and 50 (21%) with asthma (**table I**).

Concerning the subtypes of CU patients, 347 (81%) had chronic spontaneous urticaria (CSU), of whom 247 (79%) were female, 39 (9%) had isolated chronic inducible urticaria (CIndU) and 43 (10%) had CSU with CIndU (p < 0.0001). Of the patients with only CIndU, nearly half of them (42%, 16 patients) had cold urticaria, whereas the minority presented with solar urticaria (5%, 2 patients) and heat urticaria (2%, 1 patient) (p = 0.001).

Figure 1 *- Referral sources to urticaria outpatient clinic.*



Forty-three (10%) patients presented CSU with CIndU associated, the majority of which had delayed pressure urticaria (71%, 31 patients), followed by symptomatic dermographism (13%, 6 patients) ($p < 0.0001$) (**table II**). The represented P-values refer to the differences in the proportions of chronic urticaria subtypes and were obtained by chi-square goodness-of-fit test.

A laboratory profile, including thyroid laboratory tests, was requested for all patients, nevertheless 53 did not yet perform them. Of the 376 that performed the laboratory profile, 30 (8%) presented alterations in TSH and FT4, compatible with hypothyroidism in 19 (5%) and hyperthyroidism in 3 (1%). Forty-one (11%) had positive thyroid autoantibodies. All the group with hypothyroidism started daily levothyroxine sodium replacement. The clinical response of urticaria to levothyroxine sodium treatment was good in 7 (37%) patients in which urticaria became total controlled and partial in 3 (16%); conversely, 9 (47%) patients showed no improvement in clinical score ($p = 0.23$). Nevertheless, these nine patients demonstrated normalization of thyroid function after 4-6 weeks of levothyroxine sodium treatment. The patients with hyperthyroidism were medicated with anti-thyroid drugs. The clinical response of urticaria was evaluated until the normalization of thyroid function after treatment with anti-thyroid drugs. Although thyroid hormone levels were normalized after 6-8 weeks of treatment, none of the three patients showed any improvement of their respective urticaria.

In what concerns other laboratory parameters, ANA were positive in 42 (11%) patients and increased values of CRP in 31 (8%), ESR in 46 (12%) and d-dimers in 35 (9%). When considering each of these results and the patients with UAS7 > 15 (131 patients, 35%) at time of blood sampling, which translates a moderate to severe disease activity, we have verified that ANA were positive in 35 (83%) of these patients and increased values of CRP were observed in 26 (84%), ESR in 40 (87%) and d-dimers in 18 (51%), respectively. On the other hand, UAS7 > 15 was found in 96 (29%) ANA negative patients, 105 (30%) patients with normal values of CRP, 91 (28%) patients with normal values of ESR and 113 (33%) patients with normal levels of d-dimers. When comparing these variables, we verified that ANA positive

Table I *- Demographic and clinical characterization of chronic urticaria patients.*

Characteristics	**CU patients (n = 429)**
Gender, n (%)	
Male	94 (22%)
Female	335 (78%)
Mean age, years (range)	
At the onset of symptoms	43.7 (6-88)
At diagnosis	46.7 (6-88)
Average diagnostic delay	3 (0-25)
Time of disease before diagnosis, n (%)	
< 1 year	129 (30%)
1-2 years	99 (23%)
2-5 years	107 (25%)
> 5 years	94 (22%)
Median follow-up period in the outpatient clinic, years (range)	1.7 (0.1-2.75)
Atopic comorbidities, n (%)	236 (55%)
Allergic rhinitis	142 (60%)
Asthma	50 (21%)
Drug allergy	21 (9%)
Food allergy	14 (6%)
Atopic dermatitis	9 (4%)
Autoimmunity tests, n positive result/total (%)	
Autologous serum skin test	24/76 (32%)
Basophil activation test	13/38 (34%)
Helicobacter pylori infection, n positive result/total (%)	110/291 (45%)
Eradication, n (%)	96 (87%)
Reported improvement, n (%)	57 (59%)
UAS7 < 6, n (%)	316 (81%)

CU: chronic urticaria; UAS: urticaria activity score.

Table II *- Chronic urticaria diagnosis of studied population according to the EAACI/GA²LEN/EDF/WAO guidelines.*

Chronic Urticaria subtypes	CU patients (n = 429), n (%)
Isolated chronic spontaneous urticaria	347 (81%)
Chronic inducible urticaria	39 (9%)
Cold urticaria	16 (42%)
Cholinergic urticaria	9 (23%)
Symptomatic dermographism	8 (21%)
Delayed pressure urticaria	3 (7%)
Solar urticaria	2 (5%)
Heat urticaria	1 (2%)
Chronic spontaneous urticaria + Chronic inducible urticaria	43 (10%)
Delayed pressure urticaria	31 (71%)
Symptomatic dermographism	6 (13%)
Cold urticaria	3 (8%)
Cholinergic urticaria	2 (5%)
Delayed pressure urticaria + Symptomatic dermographism	1 (3%)

All data are presented as frequencies and percentages. The chi-square goodness-of-fit test indicated that the differences in the proportions of chronic urticaria subtypes diagnosed in the study were statistically significant ($p \leq 0.001$). CU: chronic urticaria.

patients demonstrated a statistically significant more severe disease than ANA negative patients ($p < 0.0001$), as well as patients with elevated values of CRP ($p < 0.0001$), ESR ($p < 0.0001$) and d-dimers ($p = 0.04$) than patients with normal levels, respectively. Seventy-six patients underwent ASST which was positive in 24 (32%) and 38 underwent BAT which was positive in 13 (34%). Of the patients who demonstrated a positive ASST, 18 (75%) presented UAS7 > 15 at time of blood sampling, in contrast with 3 (7%) ASST negative patients ($p < 0.0001$). When analyzing BAT positive patients, 11 (85%) presented UAS7 > 15, as opposed to 5 (20%) BAT negative patients ($p = 0.0003$).

In our CSU population, non-steroidal anti-inflammatory drugs (NSAIDs) trigger urticaria in 27 (7%) patients. When considering this population and UAS7 values > 15 we have found that 21 (78%) NSAID-intolerant CSU patients presented it, as opposed to 53 (15%) NSAID-tolerant CSU patients ($p < 0.0001$). *Helicobacter* (*H. pylori*) infection was tested in 291 patients and identified in 110 (45%). With the first therapeutic attempt, eradication was achieved in 96 of those patients (87%) and 57 (59%) reported urticaria improvement ($p = 0.07$) (**table I**).

At the moment of the study, 204 (48%) of CU patients were medicated with a second-generation H1-antihistamine (sgAH) daily, 99 (23%) with sgAH up to four times the standard dose, 126 (29%) with omalizumab and none with cyclosporine. Additionally, 7 (2%) patients were completing a 10-day short course of systemic corticosteroids (20 mg oral prednisolone) for management of a disease exacerbation at the time of data analysis. Of these, all of them were under sgAH four times the standard dose and five of them also under omalizumab. They were all controlled (UAS7 < 6) before this exacerbation.

Discrimination of therapeutic modalities in CU subtypes (isolated CSU, CSU with CIndU and isolated CIndU) are represented in **table III**. It was observed a statistically proven major use of sgAH daily in the group of patients with isolated CInDU and a trend to upgrade treatment (updosing sgAH or adding omalizumab) in CSU patients with or without CInDU associated ($p < 0.0001$).

UAS7 in the four weeks prior to the study date was < 6 in 316 of CSU patients, which translates a disease control of 81% (**table II**). When considering uniquely the group of CSU patients under treatment with omalizumab, disease control was accomplished in 107 patients (89%).

Discussion

In our study, we have described a large cohort of patients with CU (n = 429) followed-up at a specialized urticaria outpatient clinic of a UCARE, in a period of time of 2 years and 9 months. Demographic, clinical, laboratory and therapeutic profile of these patients were reported.

We have verified that the majority of these patients (n = 310, 65%) were referred by general practitioners. Subsequently, 67 patients (14%) were referred by other immunoallergologists. These 67 patients were referred because they failed to achieve control with sgAH treatment and needed evaluation in a specialized urticaria consultation to start third-line therapy, not everywhere available. As observed, in our population, more than half of the patients were referred by general practitioners directly, rather than other immunoallergologists. We assume this might be due to the fact that, in our country, they represent the primary health care and frequently the first medical contact, regardless of the severity of the disease, allowing direct referral to our specialized outpa-

Table III *- Discrimination of pharmacological treatment in chronic urticaria subtypes (isolated CSU, isolated CIndU and CSU+CIndU).*

Pharmacological treatment	CSU patients (n = 347)	CIndU patients (n = 39)	CSU+CIndU patients (n = 43)	P-value
SgAH 1/day, n (%)	163 (47%)	29 (74%)	12 (28%)	< 0.0001*
SgAH 2 to 4 times the standard dose	83 (24%)	4 (10%)	12 (28%)	
Omalizumab	101 (29%)	6 (16%)	19 (44%)	
Cyclosporine	0 (0)	0 (0)	0 (0)	

CSU: chronic spontaneous urticaria; CIndU: isolated chronic inducible urticaria; sgAH: second-generation H1-antihistamine. *Chi-square test; value for all the comparisons in the table.

tient clinic. Furthermore, as previously mentioned and below explained, patients may also be referred to our outpatient clinic by the emergency department, other hospitals or other specialties. Despite it, we have verified that sometimes these patients do not have a severe disease. Emergency department (n = 48, 10%) and dermatology consultations (n = 33, 7%), were also a common origin of these patients, but the data also reported a referral of colleagues from other specialties, namely internal medicine, rheumatology, pediatrics, hematology and nephrology. The wide variety of specialties through which these patients were referred shows a significant portion of physician visits due to urticaria and thus its importance in everyday practice among many specialties.

Nearly all of the evaluated patients had CSU (n = 390, 91%), of whom 43 (10%) had CIndU associated.

Maurer *et al.* descriptively compared some of these aspects among CU patients in many countries residing in Europe (EU) and Central and South America (C/SA). Among patients with CSU, CIndU was a comorbid disease in 30% of C/SA patients but only 22% of EU patients (13). Our findings show a slight lower percentage of CIndU compared with the European patients mentioned in this report. We presume that it might have been a significant lesser referral of patients with isolated CIndU, taking into consideration the high percentage of referral of patients to our center by other specialties and a probable minor awareness of CIndU disease, either by doctors or by patients who do not seek medical attention.

As previously described, 39 (9%) of CU patients had isolated CIndU, namely 16 (42%) cold urticaria, 9 (23%) cholinergic urticaria, 8 (21%) symptomatic dermographism, 3 (7%) delayed pressure urticaria, 2 (5%) solar urticaria and 1 (2%) heat urticaria. CIndU is characterized by itchy wheals, flare-type skin reactions, and/or angioedema induced by external physical factors. Our findings are similar to a report of Abajian and colleagues, who estimated the prevalence of CIndU is 13.1-14.9% among patients with chronic urticaria (14).

The demographic characteristics of our cohort, namely median age and a predominance of the female gender in CSU patients, are in line with other studies, including the Portuguese AWARE Study and consequently with German and Scandinavian AWARE patients (15, 16). This consistently reported female predominance is not explained, neither mechanistically nor clinically (15).

The patients were diagnosed with three years of average time of disease and after being observed/treated by several specialists (as about 22% presented symptoms for more than five years), demonstrating the complexity in appropriate diagnosis and management of this disease.

Regarding allergic co-morbidities, our results are concordant with others, who have reported associations between CU and atopic conditions, such as atopic dermatitis, allergic rhinitis and asthma, even though in our study in a lower percentage. Nassif *et al.* reported that more than 90% of CU patients had a personal history of atopic disease (17). In our study it was documented in 55%. Two recent cross-sectional studies of 11,217 and 12,185 patients, respectively, observed significant associations of CU with asthma, atopic dermatitis and allergic rhinitis (18, 19). Accumulating evidence shows that CU and atopic conditions are associated with aberrant immune function. Its association may reflect aberrant crosstalk between T-helper cells and mast cells (20), even though there are studies which do not support these conjectures (21). We assume that the interrelationships between these conditions are possibly complex and require further study.

In terms of laboratory findings, we found alterations in TSH and T4, compatible with hypothyroidism in 19 (5%) and hyperthyroidism in 3 (1%). Kolkhir *et al.* performed a systematic review and found hypothyroidism in 0-42.6% and hyperthyroidism in 0-17.6% of CSU patients. In this review, it was also reported that in CSU hypothyroidism is more common than hyperthyroidism (22), which is in line with our findings. In our population, thyroid autoantibodies serum levels were high in 11% (n = 41) patients, similar with a review of 24 studies and ≥ 100 patients that demonstrated that the frequency of elevated IgG thyroid autoantibodies varied from 3.7% to 37.1% (23).

Forty-one (11%) had positive thyroid autoantibodies. Of the group of patients with hypothyroidism they all started daily levothyroxine sodium replacement. The clinical response of urticaria to levothyroxine sodium treatment was good in 7 (37%) patients in which urticaria became total and partial controlled in 3 (16%). Nine (47%) patients showed no improvement in clinical score, even though they demonstrated normalization of thyroid function after 4-6 weeks of levothyroxine sodium treatment. The patients with hyperthyroidism (n = 3, 1%) were

under anti-thyroid drugs. None of the three patients showed any improvement of their respective urticaria, although thyroid hormone levels were normalized after 6-8 weeks of treatment.
According to the systematic review of Kolkhir and colleagues, in some studies treatment of hypothyroidism and hyperthyroidism led to improvement or remission of CSU in 28% and 67% of patients, respectively. However, in other, neither replacement treatment with levothyroxine nor anti-thyroid drugs had effect on improving urticaria control. The data on the efficacy of treatment with thyroid drugs including levothyroxine in CSU are not consistent. Conflicting evidence may be explained by the various confounding factors and limitations such as the small numbers of patients included in some studies and the absence of appropriate controls (22).
In what concerns other laboratory parameters, ANA were positive in 42 (11%) patients, CRP in 31 (8%), ESR in 46 (12%) and d-dimers in 35 (9%).
Antinuclear antibodies (ANA) are a group of autoantibodies directed against corresponding antigens in the nucleus and are found in many patients with systemic or organ-specific autoimmune disorders. According to Viswanathan and colleagues the percentage of CSU patients having a positive test for ANAs (titer more than 1:160) is approximately 29% (24). In our setting, positive ANA is observed in 12%. Costa and colleagues showed that the presence of ANA has been identified in patients with CU in percentages ranging from 0 to 29%. It is also referred that percentages varying between 22.6% and 84.6% are also found in apparently healthy patients or with other pathologies (8). Although measuring ANA serves as a nonspecific marker of systemic autoimmunity in rheumatologic disorders, its relationship with CSU is poorly understood. Nevertheless, Viswanathan and colleagues established association between the presence of ANA and the severity of urticaria (24). We have found, in our population, positive ANA patients had a significant more severe disease, when compared to patients with negative ANA.
ESR, CRP or d-dimer levels have been shown to be high in CSU and might correlate with disease activity as evidenced in several studies from different centers (25). In our study, we have verified that patients with elevated values of these laboratory parameters demonstrated, respectively, a statistically significant more severe disease, than patients with normal values. However, the problem remains as whether these substances are specific enough for urticaria, as they can often be elevated in many other diseases that are often co-morbid in CSU patients or even an underlying cause of the CSU (*e.g.*, chronic infections, autoimmune disorders) (26).
Based on these premises and the actual literature, we assume that the referred biomarkers are particularly useful as biomarkers of disease activity, but as they are related to inflammatory and coagulation, their interpretation in CSU must always be done with caution.
The pathogenesis of CU is complex and not yet fully understood. However, central to our current understanding of this unpredictable disease is the activation and subsequent degranulation of mast cells in the skin (2).
The degranulation of mast cells in CU can be due to different mechanisms in different patients (27). CSU is considered to be an autoimmune disorder (type I and type II) in 50% of all cases (28). Reviewing literature, up to 45% of patients with CU have IgG autoantibodies directed against either IgE (5-10%) or FcεRI (35-40%). These IgG autoantibodies can bind to and cross-link FcεRI on mast cells and basophils, resulting in their activation. This is classified to be type IIb autoimmunity in CSU. In contrast to this, type I autoimmunity in CSU is characterized by the finding of IgE autoantibodies against thyroid antigens such as thyroid peroxidase (TPO) and/or auto-allergens. This autoimmune characteristic of the disease is now regularly assessed by physicians, by means of the ASST, to aid in specific diagnosis of autoimmune-related urticaria (29). Recently, Schoepke *et al.*, in the PURIST study, also suggested the inclusion of the Basophil Histamine Release Assay (BHRA) or the BAT in the diagnostic work up of CSU patients which may allow for the identification of autoimmune CSU patients in clinical practice (30).
In our cohort seventy-six patients underwent ASST which was positive in 24 (32%) and 38 underwent BAT which was positive in 13 (34%); of these, 7 patients had both ASST and BAT positive.
In a recent metanalysis of studies in Asian patients, it was observed a higher UAS7 and high risk of angioedema in ASST positive patients, suggesting an association of test positivity and disease severity (31). Furthermore, there is also evidence showing that BAT with or without the combination of ASST can identify patients with more severe CSU (32, 33). These studies are in conformity with our results which revealed that ASST positive patients and BAT positive patients demonstrated a statistically significant more severe disease than ASST and BAT negative patients, respectively. Mast cell degranulation in CSU may also result from infection-associated signals and other unknown mechanisms. In addition to these, various other non-immunological factors can prime or trigger mast cells to induce inflammatory reactions, including different drugs such as NSAIDs (34). Also, a substantial proportion, up to 40%, of patients with CSU experience exacerbations when exposed to NSAIDs (35). In the studied sample we have verified NSAIDs were a trigger to urticaria in 27 (7%) patients. Sánchez-Borges *et al.* have observed that patients with aspirin exacerbated cutaneous disease experience more severe disease when compared with CSU patients who are tolerant to NSAIDs (35). Shin *et al.* observed that patients with NSAIDS-induced urticaria have a more severe and chronic disease (36). In our population we have also demonstrated a statistically significant more severe disease in NSAID-intolerant CSU patients when compared to NSAID-tolerant CSU patients ($p < 0.0001$).
In recent years, there has been emerging literature suggesting that *H. pylori* could be involved in the pathogenesis of CU

(37). In our study, *H. pylori* infection was tested in 291 patients and identified in 110 (45%). Eradication was achieved in 96 of those patients (87%) and 57 (52%) reported remission of urticaria, which, in our study, was not statistically significant ($p = 0.07$). Our results are in line with other studies, involving 2200 participants, with a total *H. pylori* infection rate of 44.73% (984/2200). The prevalence rate of *H. pylori* infection was 49.74% in chronic urticaria group and 40.81% in controls (38). However, the association between *H. pylori* and CSU is subject to much dispute. Several studies have shown a higher prevalence of *H. pylori* infection in chronic urticaria patients and have reported remissions of skin lesions after eradication treatment (39-41). However, other investigations supported a lack of relationship between *H. pylori* infection and CU and other studies found no correlation between *H. pylori* eradication and remission of urticarial symptoms (42).

As documented and even though *H. pylori* eradication has been recommended as part of routine chronic urticaria management by multiple authors, the evidence that *H. pylori* eradication leads to improvement of chronic urticaria outcomes is weak and conflicting (43). When analyzing our own results, we also observe that the effectiveness of *H. pylori* eradication therapy in suppressing CSU symptoms was not statistically significant. For this reason we assume that *H. pylori* eradication should be individually and carefully considered, instead of a routine recommendation. We agree with Shakouri *et al.* (43) that potential harms/burdens and benefits of this therapeutic intervention and patient values and preferences must be considered before proceeding with assessment and treatment for *H. pylori* in chronic urticaria patients.

Treatment of chronic urticaria follows a standard approach with the goal of achieving complete absence of symptoms. All patients should avoid known triggers, including certain drugs such as NSAIDs and relevant triggering stimuli in the case of CIndU. However, given that this approach only results in very few cases, symptomatic treatment is recommended for nearly all patients (2). According to the current version of the EAACI/GA²LEN/EDF/WAO chronic urticaria guideline, the non-sedative second-generation H1-antihistamines (sgAH) are the first-line therapy. If continuous treatment for 2-4 weeks does not lead to adequate control of symptoms, the guideline recommends up-dosing (up to four times the standard dose), which is much more effective than standard-dose therapy and has a similar side effect profile (3). In line with these recommendations, in our cohort, and at the moment of study, 204 (48%) patients of were medicated with sgAH daily or on-demand and 105 (24%) with sgAH two to four times the standard dose (46 patients with two H1-antihistamines, 17 with three and 42 with four).

If sufficient improvement does not occur after 2-4 weeks of sgAH therapy at a higher-than-standard dose, omalizumab should be added to the regimen. As mentioned before, it has also been shown to be effective and safe in the treatment of CIndU patients, however, it is not yet approved for patients with isolated CIndU. If there is no success after six months of omalizumab therapy, off-label treatment with cyclosporine is recommended (3). In our population, 126 patients (29%) were under treatment with omalizumab due to being refractory to sgAHs. It is worth mentioning that in our country, and in accordance with the EAACI/GA²LEN/EDF/WAO chronic urticaria guideline (2), only patients resistant to second-line therapy (four daily sgAH) are accepted for treatment with omalizumab by the hospital pharmacy and therapy commissions. As a result, every CSU patient that started omalizumab was under sgAH four times the standard dose at that moment and might have been under systemic corticosteroid therapy at that moment or in the past. Despite that, when the patient achieves a complete response to omalizumab, the daily dose of sgAH is progressively reduced and, consequently, many patients under omalizumab therapy stop using sgAH.

A similar percentage of patients treated with omalizumab was found in the Portuguese population of AWARE study, an heterogeneous non-interventional study including patients recruited from 10 participating centers of immunoallergology and dermatology throughout Portugal, designed to evaluate the real world disease burden of CU patients' refractory to sgAHs standard dose treatment, at specialized urticaria centers (15). Also in comparison with this study, we have verified, along with the analysis of our UAS7 results (mentioned below), that, in our population, a higher percentage of disease control (81%) was achieved, corroborating the fact that our patients are adequately treated and in agreement with the latest guidelines whose goal is total control of urticaria symptoms. Moreover, when considering uniquely the group of CSU patients under treatment with omalizumab, disease control was accomplished in 107 patients (89%), which reinforces the importance of a proper assessment and the well-documented efficacy of this therapeutic option, not everywhere available.

In our population, six of the patients with isolated CIndU (16%), namely, two patients with delayed pressure urticaria, one with cold urticaria, one with heat urticaria, one with solar urticaria and one with cholinergic urticaria were medicated with omalizumab. Even though it has an off-label use in CIndU, these patients presented a severe disease refractory to sgAHs that justified trying omalizumab treatment. At the moment of study, we have verified that all but one experienced a complete/partial relief of symptoms.

The efficacy of cyclosporine has been confirmed in placebo-controlled trials and it should be used with caution due to its adverse events or used only in specific groups of patients (3). In our population sample there was no patient taking cyclosporine. Seven (2%) patients were completing a 10-day short course of systemic corticosteroids (20 mg oral prednisolone) for management of a disease exacerbation at the moment of data analysis. Of these, all of them were medicated sgAH four times the standard dose and five of them also with omalizumab. They were

all controlled (UAS7 < 6) before this exacerbation. This is in agreement with several guidelines and expert opinions which recommend a short course of oral corticosteroids only in exacerbations and, due to the risk of serious adverse events, do not recommend their long-term use (15).

When comparing with the Portuguese AWARE study about the real-life clinical practice setting in Portugal in which almost 11% of patients were taking oral corticosteroids (15), a lower percentage is described in our study.

The use of UAS helps to monitor the evolution of the disease and the efficacy of treatment. It is the best method for assessing activity given that this instrument evaluates the seven days prior to the medical visit, evaluating more accurately a disease that has a fluctuating course. We have considered the median UAS7 of the four weeks prior to the study date and concluded that it was < 6 in 316 CSU patients, which translates a disease control of 81%.

Our study has some limitations that must be considered. First, this study is retrospective, with analysis of patients' database. Some information, as results of laboratory tests, was not available for all patients (12%). Second, we didn't provide any data concerning follow-up or quality of life. Despite it, we provide helpful data regarding distribution of urticaria ethology, clinical course time, laboratory tests and pharmacological treatment of patients treated in a Urticaria Centers of Reference and Excellence.

Conclusions

A central aspect to be generally considered in the treatment approach and patient management is the individual disease severity. Given the prevalence of chronic urticaria, general practitioners or emergency medical services are frequently the first to be consulted, as observed in our cohort. For patients with mild disease, standard-dose antihistamine therapy is usually sufficient. Nevertheless, if this is not the case, patients should be referred to a specialist who typically have more diagnostic and treatment options at their disposal. UCAREs are certified by the European GA²LEN network and are characterized by a high degree of specialization in terms of research and clinical patient care.

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Conflict of interests

The authors declare that they have no conflict of interests.

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The spectrum of inborn errors of immunity: a single tertiary center retrospective study in Alborz, Iran

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Key words

Inborn errors of immunity; clinical features; immune system; demographic characteristics; heterogeneous disorders.

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Impact statement

The most common known immunodeficiency disease in the present study was immunoglobulin A deficiency (IgAD), severe combined immunodeficiency (SCID) and common variable immune deficiency (CVID).

Summary

Background. *Inborn errors of immunity (IEIs) are a group of heterogeneous disorders with inherited faults in the immune system that increase susceptibility to infections, malignancies, lymphoproliferation, and autoimmune/autoinflammatory disorders.* ***Methods.*** *We retrospectively studied the demographic characteristics, clinical features, and immunological profiles of the 90 IEIs patients, who were diagnosed and classified according to the European Society for Immunodeficiencies (ESID) and International Union of Immunological Societies (IUIS) criteria from July 2010 to June 2021. The study was carried out in the Non-communicable Diseases Research Center, Imam Ali Hospital, Alborz, Iran.* ***Results.*** *Within a period of 11 years, 53 (58.9%) males and 37 (41.1%) females were diagnosed and followed-up for 20 IEI disorders. The median (IQR) age of onset, age of clinical diagnosis and diagnostic delay was 0.7 (0.08-2.0), 3.18 (1.0-8.0) and 1.5 (0.17-5.0) years, respectively. Twelve patients (36.4%) had a positive family history of IEI, and the majority of patients (84.5%) had recurrent infections. Pneumonia (51.7%) was the most common clinical manifestation among IEI patients, followed by skin complications (46.2%). The most frequently diagnosed IEI was immunoglobulin A deficiency (IgAD) (14.4%) and severe combined immunodeficiency (SCID) (11.1%). Predominantly antibody deficiencies group (36.7%) was the most common category, followed by combined immunodeficiencies with associated or syndromic features group (27.8%).* ***Conclusions.*** *IEIs have different patterns within populations with high consanguinity. There is a need to searching for underlying genetic and epigenetic factors in most common IEIs in Alborz.*

Introduction

Inborn errors of immunity (IEIs) is considered a heterogeneous group of more than 400 inherited disorders, leading to qualitative or quantitative defects in immune system components (1, 2). Patients with IEI are generally prone to recurrent and persistent or unusual serious infections and some have a tendency to immune dysregulation (3). They have a widespread phenotype with often high rates of mortality and morbidity, making the diagnosis and treatment challenging (4). IEI clinical manifestations include, but are not limited to, recurrent infections, autoimmune/inflammatory diseases, enteropathy, failure to thrive, allergy, lymphoproliferation and/or malignancy (5). During the last decade, recent progress in genetic research and immunological finding has allowed a greater understanding of pathomechanisms underlying IEIs (6). On the other hand, the development and use of diagnostic techniques, especially flow cytometry analysis and next-generation sequencing, significantly have helped facilitate the diagnosis of IEIs (7). However, general practitioners/pediatricians as the first encounterers may not be able to recognize patients suspected of IEI due to the lack of training and awareness, which is the main reason for delayed diagnosis or misdiagnosis and inadequate treatment, which leads to unfavorable consequences (8, 9).

The incidence of IEI disorders, ranges from 1:500 to 1:1,000,000, depending on the specific primary genetic defect and geographical region (10, 11). The overall predicted prevalence of IEIs is almost 1 in 1200 live births except for immunoglobulin A (IgA) deficiency, which is more common in the general population (12, 13). However, the prevalence of IEIs are supposed to be more than the world's average due to the high consanguinity rate in Iran (10).

This study aimed to report the distribution, clinical presentations, and immunologic features of 90 IEI patients living in Alborz province, Iran.

Materials and methods

Data collection

This longitudinal study was carried out in the Non-communicable Diseases Research Center, Imam Ali Hospital, Alborz, Iran. All patients with IEI, diagnosed during the period from July 2010 to June 2021, were included in the study. A total of 90 patients were included for classification and investigation based on updated diagnostic guidelines confirmed by the European Society for Immunodeficiencies (ESID) working party (14). In addition, the gathered information was entered into the data form and divided into five sections: laboratory and molecular findings, clinical manifestations, sociodemographic data, and current life status. Immunodeficiencies secondary to other conditions (*e.g.*, human immunodeficiency virus infection, malnutrition and medical treatment) were excluded as well. The study was approved by the Ethics Committee of the Alborz University of Medical Sciences (Approval code: IR.ABZUMS.REC.1399.241).

Evaluation sheet

For documentation, an evaluation sheet was developed to contain all patients' demographic data such as age, gender, age at onset of symptoms, age at diagnosis, delay of diagnosis, parental consanguinity, family history of IEI, dead or alive status and clinical manifestations. Laboratory investigations were performed using standard techniques and included complete blood count, peripheral blood lymphocyte subsets including the basic panel of T-cell subsets (CD3, CD4, CD8), B-cell (CD19, CD20), and natural killer cell (CD56/16) − assessed using flow cytometry analysis −, and measurement of serum immunoglobulins (IgG, IgA, IgM, and IgE) level − assessed using nephelometry and enzyme-linked immunosorbent assay (ELISA). If required, Nitroblue tetrazolium test (NBT), measurement of serum alpha-fetoprotein (AFP), assessment of the expression of CD18/CD11 on neutrophils by flow cytometry, complement hemolytic activity (CH50), anti-tetanus IgG, anti-diphtheria IgG, and also anti-pneumococcal antibody titer were performed. In addition, patients with incomplete data or those who did not meet the ESID criteria were excluded. Medical data were obtained after receiving written informed consent from all patients or/and their surrogates.

Statistical analysis

Information were gathered in an Excel database and were converted for analysis using the SPSS statistical software package version 25.0 (IBM corporation, Chicago, IL, USA). The Shapiro-Wilk test was used to validate the assumption of normality for a variable, and the nonparametric or parametric tests were carried out according to the normality supposal. Frequency and percentages were reported for qualitative variables and median (interquartile range, IQR) for quantitative variables. Fisher's exact test and $\chi 2$ tests were used for 2×2 comparisons of categorical variables. To compare numerical variables, the nonparametric Mann-Whitney U test was used. A P-value < 0.05 was considered statistically significant.

Results

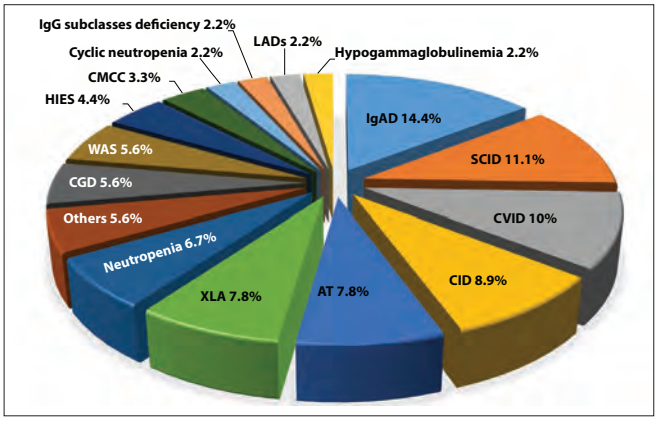
Epidemiologic characteristics of IEI patients

Totally, 90 patients with 20 types of IEIs diagnosed from July 2010 to June 2021 were enrolled in the study (**figure 1**). As shown in **figure 1**, immunoglobulin A deficiency (IgAD) was the most common IEI (13 patients (14.4%)), followed by severe combined immune deficiency (SCID) and common variable immune deficiency (CVID) in 10 (11.1%) and 9 (10.0%) patients, respectively. The median age of patients at the time of the study was 13.0 years (IQR:

4.0-24.0 years, varying from 0.2 to 46 years). The median (IQR) age of onset, age of clinical diagnosis and diagnostic delay was 0.7 (0.08-2.0), 3.18 (1.0-8.0) and 1.5 (0.17-5.0) years, respectively. The male/female ratio was approximately 1.4:1 (53 (58.9%) male and 37 (41.1%) female). At the time of the study, 54 (78.3%) patients were alive and 15 (21.7%) patients were deceased.

The detailed demographical data is summarized in **table I**. The highest age of clinical diagnosis belonged to CVID patients (median (IQR):11.96 (4.56-21.29) years) and the longest delay in diagnosis was observed in hyper IgE syndrome (HIES) patients (median (IQR): 7.0 (7.0-7.0) years). Also, the lowest delay in diagnosis and the shortest duration from diagnosis to death were found in SCID patients with a median (IQR) 0.1 (0.09-1.0) and 0.94 (0.2-18.35) years, respectively. The median (IQR) age of CVID and CID patients (23.0 (15.75-30.25) and 2.0 (0.55-3.87) years, respectively), were the highest and the lowest age at the time of the study. The detailed patient's demographical data in the ten most common IEI phenotypes are represented in **table II**. According to the ten categories of International Union of Immunological Societies criteria (IUIS), most of the patients were in the predominantly antibody deficiencies group (n = 33, 36.7%), followed by combined immunodeficiencies with associated or syndromic features (n = 25, 27.8%) and congenital defects of phagocyte number and function (n = 15, 16.7%) groups. **Table III** shows the detailed distribution of reported clinical diagnoses in the ten categories of IUIS classification. Among ninety registered patients, 25 patients (27.8%) had confirmed molecular diagnosis.

***Figure 1** - The distribution of patients with IEI diagnosis.*



The most common clinically diagnosed IEIs were IgAD and SCID. Others include HIgM, ICF syndrome, hereditary angioedema, ALPS-like, and complement deficiency. The frequency of each of them is approximately 1.1% (one person). CVID: common variable immune deficiency; SCID: severe combined immune deficiency; AT: ataxia-telangiectasia; XLA: x-linked agammaglobulinemia; IgAD: immunoglobulin A deficiency; CGD: chronic granulomatous disease; HIES: hyper-IgE syndromes; WAS: Wiskott-Aldrich syndrome; CMCC: chronic mucocutaneous candidiasis; CID: combined immune deficiency; LAD: leukocyte adhesions deficiency syndrome; HIgM: hyper immunoglobulin M; ALPS-like: autoimmune lymphoproliferative syndrome Like; ICF: immunodeficiency centromeric region instability facial anomalies syndrome.

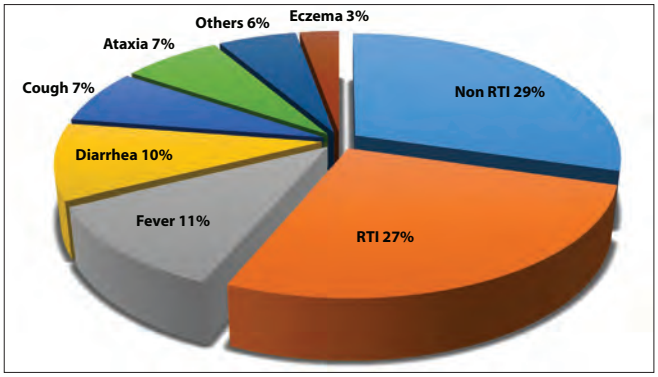
Clinical spectrum of IEI patients

In our study, 12 of 33 patients with available data (36.4%) had a positive family history of IEI. The history of infectious complications was reported in 60 patients (84.5%). Twenty-three types of clinical manifestations were reported as the first clinical presentation of IEI patients. Respiratory tract infections (RTI) (27.0%), including pneumonia, sinusitis, sinopulmonary infections, otitis media, common cold, recurrent pharyngitis, and non-respiratory tract infections (nRTI) (29.0%), including urinary tract infections, skin infections, gastrointestinal infections, BCGosis, oral candidiasis, eczema and arthritis, were the most common first presentation in IEI patients (**figure 2**).

As shown in **table IV**, the most reported clinical manifestations among patients were pneumonia (51.7%), skin complications (46.2%) (include dermatitis, psoriasis, eczema and vitiligo), and otitis media (41.8%). In patients with IgAD and SCID, as the most frequent clinical diagnosis, the most common presentation was pneumonia (66.7% and 83.3%, respectively).

Nineteen cases had a history of autoimmunity and ten of them had polyautoimmunity. Most of the patients with autoimmunity had an ultimate diagnosis of IgAD (36.8%), CVID (26.3%), or ataxia-telangiectasia (10.5%). The most common autoimmune disorders were hypothyroidism and Insulin-dependent diabetes mellitus (IDDM), each was found in seven people (7.8%).

***Figure 2** - The first clinical presentation in patients with IEIs.*



The most common first presentation in IEI patients was Non RTI (29.0%) and RTI (27.0%). Others include: failure to thrive, bleeding, limb swelling and periorbital edema, each found in one person (1.5%). RTI includes: pneumonia, sinusitis, sinopulmonary infections, otitis media, cold and recurrent pharyngitis. Non RTI includes: urinary tract infections, skin infections, gastrointestinal infections, arthritis, BCGosis, oral candidisis and eczema. RTI: respiratory tract infection.

Table I *- Demographic data of patients in most categories of inborn errors of immunity.*

Parameters	Total (n = 90)	Predominantly antibody deficiencies (n = 33)	Combined immunodeficiencies with associated or syndromic features (n = 25)	Congenital defects of phagocyte number, function, or both (n = 15)	Immunodeficiencies affecting cellular and humoral immunity (n = 11)	P-value
Age, y, median (IQR)	13.0 (4.0-24.0)	21.0 (8.0-27.0)	7.0 (2.75-16.25)	9.0 (5.5-23.25)	2.0 (0.5-16.75)	**0.014***
Sex ratio, M/F	53/37	25/8	13/12	7/8	5/6	0.109
Consanguinity, %	45 (59.2)	12 (46.2)	9 (40.9)	10 (76.9)	10 (90.9)	**0.014***
Dead/Alive (%)	15/54 (21.7)	9/15 (37.5)	1/18 (5.3)	0/12 (0)	5/5 (50)	**0.003***
Age at onset, y, median (IQR)	0.7 (0.08-2.0)	1.08 (0.52-3.1)	0.25 (0.0-1.37)	0.0 (0.0-2.0)	0.3 (0.11-1.05)	**0.043***
Age at clinical diagnosis, y, median (IQR)	3.18 (1.0-8.0)	4.0 (1.51-12.94)	2.0 (0.5-7.0)	5.0 (1.75-6.12)	0.55 (0.18-2.75)	**0.031***
Delay in diagnosis, y, median (IQR)	1.50 (0.17-5.0)	2.35 (0.44-5.07)	1.0 (0.16-6.5)	2.5 (0.37-5.37)	0.17 (0.09-1.87)	0.288
Course of disease, y, median (IQR)	12.11 (4.97-22.25)	13.95 (8.0-21.81)	6.5 (3.73-21.25)	15.5 (7.39-26.0)	1.5 (0.2-16.8)	0.125

M: Male; F: Female; N: Count; Y: Year; the median is shown with 25th and 75th percentiles; *P-value is statistically significant < 0.05.

Table II *- Demographic data of patients in the ten most common inborn errors of immunity.*

Category	No. of cases	Age, y, median (IQR)	Sex ratio, M/F	Consanguinity (%)	Age at onset, y, median (IQR)	Age at diagnosis, y, median (IQR)	Delay in diagnosis, y, median (IQR)	Course of the disease, y, median (IQR)
IgAD	13	12.0 (8.0-23.0)	9/4	37.5	0.29 (0.0-3.02)	3.09 (0.79-5.0)	1.7 (0.09-5.0))	11.4 (8.0-17.45)
SCID	10	2.0 (0.5-18.0)	4/6	90	0.3 (0.11-0.75)	0.4 (0.16-1.64)	0.1 (0.09-1.0)	0.94 (0.2-18.35)
CVID	9	23.0 (15.75-30.25)	5/4	75	4.5 (2.0-14.69)	11.96 (4.56-21.29)	1.96 (0.43-7.06)	14.45 (4.25-18.75)
CID	8	2.0 (0.55-3.87)	4/4	50	0.18 (0.04-0.80)	0.75 (0.16-N/A)	N/A	3.34 (2.0-N/A)
AT	7	13.0 (10.5-27.0)	1/6	66.7	1.0 (0.3-2.0)	4.0 (2.0-9.0)	4.0 (0.2-8.5)	13.0 (9.0-25.85)
XLA	7	22.0 (4.0-26.0)	7/0	28.6	0.7 (0.6-1.0)	3.0 (1.35-5.80)	2.3 (1.1-5.1)	21.75 (3.4-25.3)
Neutropenia	6	8.0 (2.1-22.5)	2/4	100	0.0 (0.0-3.0)	4.0 (2.25-5.75)	2.5 (1.25-4.5)	8.0 (8.0-N/A)
CGD	5	16.5 (7.0-23.7)	1/4	75	0.0 (0.0-N/A)	5.75 (5.0-12.87)	6.5 (0.0-N/A)	23.0 (5.58-N/A)
WAS	5	5.0 (3.5-25.0)	5/0	20	0.05 (0.0-2.27)	0.65 (0.19-5.50)	0.60 (0.17-3.25)	4.95 (2.72-28.25)
HIES	4	28.0 (8.0-N/A)	2/2	0	0.0 (0.0-0.0)	7.0 (7.0-7.0)	7.0 (7.0-7.0)	N/A

M: Male; F: Female; Y: Year; the median is shown with 25th and 75th percentiles; IgAD: immunoglobulin A deficiency; SCID: severe combined immune deficiency; CVID: common variable immune deficiency; CID: combined immune deficiency; AT: ataxia-telangiectasia; XLA: x-linked agammaglobulinemia; CGD: chronic granulomatous disease; WAS: Wiskott-Aldrich syndrome; HIES: hyper-IgE syndromes.

Immunological findings of IEI patients

White blood cell (WBC) in 47 (63.5%) and lymphocyte counts in 43 (79.6%) patients were within the normal range, while lymphopenia and lymphocytosis were reported in 5.6% and 14.8% of patients, respectively. **Table V** shows the spectrum of immunological findings in the study population.

The total count of lymphocyte subsets including CD3$^+$, CD8$^+$, CD19$^+$ and CD20$^+$ were within the normal range in 24 (41.4%), 30 (50.8%), 23 (44.2%), and 14 (56.0%) patients, respectively, while a decreased number of CD4$^+$ T cells was reported in 31 (52.5%) patients. The majority of patients with IEIs had a normal range of IgM and IgE (55.0% and 58.0%, respectively), while low IgG and IgA serum levels were reported in 47.5% and 50.7%, respectively.

In predominantly antibody deficiencies as the largest group (36.7% of patients), lymphocytosis was reported in most of the patients (11, 44.0%) and a high rate of CD8$^+$ T cells was

Table III *- Distribution of IEIs according to the 2019 Update of the International Union of Immunological Societies (IUIS) Phenotypical classification.*

Type	Number of cases (%)
Immunodeficiencies affecting cellular and humoral immunity	11 (12.2)
SCID	10
HIgM	1
Combined immunodeficiencies	25 (27.8)
CID	8
AT	7
WAS	5
HIES	4
ICF1	1
Predominantly antibody deficiencies	33 (36.7)
IgAD	13
CVID	9
XLA	7
IgG subclass deficiency	2
Hypogammaglobulinemia	2
Diseases of immune dysregulation	1 (1.1)
ALPS-like	1
Congenital defects of phagocyte number, function, or both	15 (16.7)
Neutropenia	6
CGD	5
Cyclic neutropenia	2
LADs	2
Defects in Intrinsic and Innate immunity	3 (3.3)
CMCC	3
Auto-inflammatory disorders	0 (0)
Complement deficiencies	2 (2.2)
Complement deficiency	1
Hereditary angioedema	1
Bone marrow failure	0 (0)
Phenocopies of IEI	0 (0)

reported in 9 (50.0%) patients; also the majority of patients were reported to have a low level of IgG, IgM and IgA (59.1%, 47.6%, and 64.3%, respectively). In the Combined immunodeficiencies with associated or syndromic features group (27.8% of patients), the frequency of $CD3^+$ and $CD4^+$ T cells were lower than the normal range in the majority of patients (50.0% and 55.0%, respectively). Moreover, low serum level of IgA was reported in 47.8% of the patients.

Discussion

IEI disorders are a heterogeneous group of genetic disorders associated with severe and recurrent infections, autoimmune diseases, and increased occurrences of malignancies (5). Although an increase in the number of specialists in the field of clinical immunology and increasing knowledge of practitioners have improved early diagnosis and management of this significant and rare group of disorders (15), IEIs are still underdiagnosed and there is a noteworthy diagnosis lag even in developed countries (9). Moreover, due to the lack of facilities, diagnosing IEIs continues to be a challenge in developing countries (6). It is worth mentioning that delayed diagnosis and misdiagnosis mainly reflect the poor knowledge about IEI among general practitioners/pediatricians. In this study, the median diagnostic delay was 1.5 (0.17-5.0) years, which was less than the previous cohort of 98 Iranian patients (6.1 years) in 2016 (4) and the Middle East and North Africa (MENA) Registry (3.4 years) (16). However, it was longer than the reported diagnostic delay (10 months) in the latest update on the Iranian National Registry of Primary Immunodeficiencies (10), as well as recent surveys in Pakistan (6) Oman (17), and Kuwait (18). In the present study, the diagnostic delay in HIES patients was longer than other PID patients. The first clinical manifestations are varied in HIES patients, so, the diagnostic delay may be high and they have been seen by a clinical immunologist too late. It is worth mentioning that in HIES patients, allergic reaction and elevated serum IgE level is one of the main manifestations that sometimes lead to misdiagnosis with an allergy and is treated as an allergic patient for years by non-immunologists. The longer the diagnostic delay, the greater and the worse long-term complications such as bronchiectasis, which per se lead to significant mortality and morbidity. Early recognition and prompt diagnosis of IEIs by raising the index of physicians' suspicion of these disorders helps in limiting significant disease-related mortality and morbidity and improves the patients' quality of life (19). Registration of Iranian IEI patients might have performed the main role in reducing the diagnostic delay since it raised the knowledge of medical staff about such disease. Of note, the available screening test for IEI is insufficient both at the national level and the nearby medical centers. As a consequence, some severe forms of IEI such as SCID might have died during infancy from severe infections before a definitive diagnosis is made (20).

In the present research study, the most common IEIs were IgAD, SCID and CVID. Therefore, the proportion of patients with IEI, who are are difficult to diagnose and prone to severe complications, has significantly raised. Previous cohort studies on IEI indicated that approximately all patients with IEI had

Table IV *- Clinical manifestations of inborn errors of immunity patients with full follow-up.*

Parameters	Total n (%)	Predominantly antibody deficiencies (%)	Combined immunodeficiencies with associated or syndromic features (%)	Congenital defects of phagocyte number, function, or both (%)	Immunodeficiencies affecting cellular and humoral immunity (%)	P-value
Pneumonia (n = 60)	31 (51.7)	58.3	44.4	16.7	71.4	0.192
Sinusitis (n = 54)	22 (40.7)	73.9	12.5	16.7	20	**0.001***
Otitis media (n = 55)	23 (41.8)	41.7	53.3	28.6	60	0.634
Bronchiectasis (n = 51)	5 (9.8)	23.8	0	0	0	0.079
Skin complications (n = 52)	24 (46.2)	22.2	72.2	40	16.7	**0.012***
Meningitis (n = 46)	4 (8.7)	6.3	12.5	0	0	0.720
Oral candidiasis (n = 52)	13 (25.0)	5.9	22.2	16.7	50	0.131
Septic arthritis (n = 42)	4 (9.5)	7.7	12.5	0	20	0.778
Malignancy (n = 54)	1 (1.9)	0	6.3	0	0	0.547
Hepatomegaly (n = 55)	14 (25.5)	36.4	0	42.9	14.3	**0.041***
Splenomegaly (n = 55)	11 (20)	30.4	6.3	16.7	0	0.157
Lymphadenopathy (n = 52)	18 (34.6)	42.1	23.5	83.3	0	**0.015***
Autoimmunity (n = 60)	19 (31.7)	40	28.6	0	20	0.253
Enteropathy (n = 42)	8 (19)	42.9	0	0	20	**0.022***
Clubbing (n = 51)	7 (13.7)	22.7	0	40	0	0.075
Failure to thrive (n = 54)	14 (25.9)	28	14.3	20	50	0.410
Conjunctivitis (n = 46)	6 (13.0)	33.3	0	0	0	**0.028***
Asthma/Allergy (n = 54)	15 (27.8)	20	33.3	25	33.3	0.793

*P-value is statistically significant < 0.05; n: number; skin complications include: psoriasis, vitiligo, rash, vasculitis and alopecia.

a history of recurrent infection before diagnosis was finalized (21, 22). In the current study also, 84.5% of patients represented different infections among which, pneumonia (51.7%) was the most common clinical manifestation in patients with IEIs that followed by skin complications (46.2%) and otitis media (41.8%). In this regard, a study in a single-center pediatric hospital in Northern Iran for 21 years represented pneumonia as the most frequent infectious manifestation in IEI patients (23). In another study in a single tertiary care center in China, the results of the clinical manifestations distribution were similar to the present study, in which respiratory infection including pneumonia was the most common complication (79.5%) and the second common complications were infections of the skin and mucous membranes (33.9%) (5). Other studies from Egypt (24) and Pakistan (6) presented almost the same results. Dermatological manifestations are common in IEIs and have been reported in up to 48% of patients with any of these pathologies (25). In the present study, skin complications was the second most common clinical manifestation (46.2%), almost similar to what is reported in the literature. Most of the patients with confirmed IEI present otitis as one of the recurrent infections (26). In the current study, the third most common clinical manifestation was otitis media (41.8%). Differences in the prevalence of clinical manifestations among different countries may be linked to the differences in data collection methods with the variable degrees of expertise and diagnostic facilities. However, poor availability and unaffordability of medicines might have made these severe complications much more prevalent among our patients. Patients with IEIs are frequently diagnosed based on a clinical history of recurrent infections due to less virulent or atypical pathogens (6), though, they can also present with non-infectious manifestations, such as autoimmune diseases

Table V *- Immunological findings of patients with inborn errors of immunity.*

Parameters	Total (n = 90)	Predominantly antibody deficiencies (n = 33)	Combined immunodeficiencies with associated or syndromic features (n = 25)	Congenital defects of phagocyte number, function, or both (n = 15)	Immunodeficiencies affecting cellular and humoral immunity (n = 11)	P-value
WBC × 10^3 (cell/μL), median (IQR) (n = 74)	7.550 (4.700-10.892)	8.800 (6.975-11.050)	8.400 (5.647-11.155)	4.070 (3.712-9.000)	5.400 (4.000-10.080)	**0.034***
Absolute lymphocytes counts × 10^3 (cells /μL), median (IQR) (n = 72)	2.603 (1.750-4.270)	3.312 (2.105-6.333)	2.112 (1.466-4.700)	2.477 (1.740-2.968)	2.052 (0.720-3.713)	0.148
Absolute neutrophils counts × 10^3 (cells /μL), median (IQR) (n = 68)	4.024 (1.848-6075)	4.071 (2.376-5.766)	4.335 (3.262-6.600)	1.768 (0.516-5.341)	2.268 (0.969-5.833)	0.147
$CD3^+$ T cells percentage, (n = 56)	70.0 (50.38-79.0)	74.0 (61.0-80.0)	64.7 (46.7-79.0)	79.7 (71.0-82.5)	12.0 (1.5-61.5)	**0.005***
$CD4^+$ T cells percentage (n = 57)	32.0 (17.4-4.25)	35.5 (24.0-40.1)	32.8 (20.0-41.0)	36.0 (29.7-49.0)	2.0 (0.8-13.5)	**0.004***
$CD8^+$ T cells percentage (n = 57)	30.0 (15.95-38.0)	32.0 (20.0-49.0)	30.0 (19.8-33.0)	30.0 (13.0-36.8)	11.1 (1.1-34.3)	0.139
$CD19^+$ percentage (n = 54)	15.5 (3.85-25.34)	6.0 (0.0-16.0)	18.0 (5.0-33.9)	10.0 (7.9-17.0)	50.0 (8.0-83.6)	**0.016***
$CD20^+$ percentage (n = 27)	18.0 (6.0-39.3)	16.7 (8.5-23.0)	21.4 (7.5-46.8)	9.5 (5.1-13.4)	43.0 (13.5-73.6)	0.180
IgG, (mg/dL), median (IQR) (n = 65)	464.0 (180.0-1135.5)	420.0 (97.5-1017.7)	672.0 (170.0-1203.0)	844.0 (471.5-2911.5)	211.0 (51.75-363.0)	**0.025***
IgA (mg/dL), median (IQR) (n = 70)	13.5 (3.2-73.77)	7.0 (0.0-18.0)	30.0 (1.0-156.0)	77.0 (15.25-257.0)	28.0 (6.25-64.0)	**0.018***
IgM (mg/dL), median (IQR) (n = 64)	57.0 (22.0-156.75)	40.0 (10.0-96.5)	90.0 (43.0-256.0)	156.0 (34.5-271.0)	35.5 (7.25-56.0)	**0.033***
IgE (mg/dL), median (IQR) (n = 52)	5.65 (1.0-165.0)	4.0 (0.0-10.0)	24.4 (1.02-796.75)	22.5 (10.2-587.5)	84.9 (0.0-165.75)	0.198

Ig: Immunoglobulin; WBC: white blood cell; the median is shown with 25^{th} and 75^{th} percentiles; *P-value is statistically significant < 0.05.

(27). Autoimmunity has often been recognized in connection with different forms of IEI (28). It is worth mentioning that, autoimmune disorders may be the first manifestation of the disease in some PID patients such as CVID (29). In the current study, autoimmunity was observed in 31.7% of patients while in patients with IgAD and CVID, the prevalence of autoimmunity was reported in 58.3% and 62.5% respectively. The frequency of autoimmunity in CVID patients (62.5%) was higher than other forms of predominantly antibody deficiencies as the largest IUIS group which is similar to previous studies (30).
Recently, the overall prevalence of autoimmunity in CVID patients was reported to be 29.8% in a systematic review study (31). Another study from Brazil reported autoimmune diseases in 61.5% of patients diagnosed (32) but in another study on Tunisian patients, the prevalence of autoimmunity was reported in 6.8% of IEI patients (33). One of the reasons for the difference between our data and other studies in the frequency of autoimmunity may be the difference in the study design. In the previous studies, the presence of immunodeficiency in patients with pure autoimmunity was investigated and identified several immunodeficient patients who were primarily diagnosed with pure autoimmune disorders (34).
Our data further showed that consanguinity and family history of IEI, seen in 59.2% and 36.4% respectively, were the most predictive factors for IEI diagnosis especially in a disease with an autosomal recessive pattern of inheritance (35). It is noteworthy that consanguinity has also been recognized as an important relevant factor for the high incidence of SCID, AT, and CGD, reported in Iran (36-38) and SCID in Kuwait (39).

The first report of IEI registry of Iran was published in 2002 consisted of 440 IEI patients (40). Recently, Iranian immunologists have published the 20-year survey of the IEI registry from the recently structured national IEI network organizing 31 collaborating hospitals affiliated to 26 medical science universities from the main provinces (10). In those surveys, 3056 patients were registered, while the majority were diagnosed with primary antibody deficiency (PAD) (29.5%). Based on the IUIS classification system, in our study, the most prevalent groups were predominantly antibody deficiencies (36.7%), combined immunodeficiencies with associated or syndromic features (27.8%), and congenital defects of phagocyte number, function or both (16.7%) groups. But in the fourth update on the Iranian National Registry of Primary Immunodeficiencies in 2018, among the newly diagnosed IEI patients, the autoinflammatory disorders group were the most common group (31.4%), followed by predominantly antibody deficiencies (22.2%) and combined immunodeficiencies with associated or syndromic features (18.6%) (10). In another study of 528 Indian children, the most common groups were Immunodeficiencies affecting cellular and humoral immunity and Congenital defects of phagocyte number, function, or both which accounted for 29% of all patients (41). In another study in Pakistan (42) and Egypt (43), Combined immunodeficiencies with associated or syndromic features (36.6%) and Immunodeficiencies affecting cellular and humoral immunity (30.0%) groups, were the most common groups respectively. It is important to know the prevalence of IEI groups among different countries to increase awareness, promote optimal treatment, support research in the field of disorders of immunity and facilitate recognition.

It would be quite challenging to estimate a total number of undiagnosed and unregistered IEI patients. This number, therefore, does not show the frequency and the burden of IEI in our study community. This is due to multiple limiting factors. First of all, it did not include patients with mild manifestations, who are usually managed as outpatients by general practitioners, pediatricians, or other specialists at various health centers and private clinics in the country. Also, asymptomatic IEI patients were not included in the study. Moreover, the present study contains some limitations, including limited molecular diagnostic data and small sample size. As the number of clinical immunologists and access to diagnostic tests at our center increased, we hope for a reduction in delayed diagnosis and early diagnosis of more patients in future years.

Conclusions

In conclusion, our study describes a sample of Iranian patients having a variety of IEIs with a high frequency of predominantly antibody deficiencies. Pneumonia and inflammatory skin complications showed widespread involvement as the clinical manifestations whereas pneumonia and fever were the most-frequent first presentation of IEIs. Physicians should suspect immunodeficiency disorder in patients with a history of recurrent infections and/or in complicated patients with inflammatory diseases that do not respond to treatment.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Analysis of adrenaline autoinjectors acquisition in Portugal over 15 years

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Key words

Adrenaline autoinjector; anaphylaxis; acquisition; prescription; prevalence.

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Impact statement

The acquisition of adrenaline injectors could be a predictor in the prevalence of patients at risk of anaphylaxis.

Summary

Background. *The adrenaline autoinjector (AAi) is universally recommended as the first-line treatment for anaphylactic reactions occurring outside the medical setting. The quantification of its acquisition may help estimate the prevalence of patients at risk of anaphylaxis with an indication for AAi.* ***Aims.*** *Evaluation of the global and regional frequency of AAi purchases in Mainland Portugal between 2003-2017 and calculate the inherent costs in 2017.* ***Methods.*** *AAi acquisition distribution analysis along this period. The population was divided in two age groups according to the adrenaline dosage.* ***Results.*** *A total of 10,993 AAi units of 0.15 mg/0.3 mL and 28,619 of 0.3 mg/0.3 mL were acquired in these 15 years, with an annual average of 733 and 1908 units, respectively. In cumulative values terms, Lisbon showed the highest number of AAI acquired and higher prevalence per region/100,000 inhabitants in both groups. In 2017, the annual cost for each age group was €64,202.71/€187,447.70 for patients and €37,706.35/€110,113.30 for the National Health System.* ***Conclusions.*** *In the last 15 years, there was a progressive increase in AAi acquisition. We estimate a rate of anaphylaxis occurrence in Portugal according to AAi acquisition of 0.165%.*

Introduction

Anaphylaxis, according to the World Allergy Organization (WAO), is defined as a potentially fatal severe systemic hypersensitivity reaction of sudden onset after exposure to an allergen (1, 2). Since it is a medical emergency, early treatment is critical. Intramuscular adrenaline is the first-line treatment for this emergency, and its dosage should be adjusted according to the patient weight (3, 4). Given that most episodes of anaphylaxis occur in the community, *i.e.*, outside the medical setting, the adrenaline autoinjector (AAi) should be prescribed to patients at risk of anaphylaxis recurrence (5, 6).

The European Academy of Allergy and Clinical Immunology (EAACI) in 2014 published the guidelines on anaphylaxis, which includes indications for the prescription of this device (7).

Existing data on the prevalence and incidence of anaphylaxis are inaccurate and correspond to default estimates since this pathology is often underdiagnosed or underreported. Based on publications between 2010 and 2015, the estimated frequency of anaphylaxis is 50-112 episodes per 100,000 inhabitants-year, estimated prevalence of 0.3% to 5.1% (8-12).

In Europe, 2 to 8 cases per 100,000 inhabitants/year have been estimated, with a growing trend, inferring that approximately 0.3% of the European population is at risk of having an episode of anaphylaxis at some point in their life (13). Some population-based studies estimated a rate of occurrence of anaphylaxis based on the acquisition of AAi ranging from 0.083% (10) to 0.95% (9), corresponding to the acquisition of 83 units of AAi and 954 units per 100,000 inhabitants in Israel and Canada, respectively.

In Portugal, based on the notified cases of anaphylaxis in the Catálogo Português de Alergias e Outras Reações Adversas (CPARA), Amaral *et al.* (14) recorded 1209 cases of anaphylaxis during a period of 10 months in a total of 20,389 records, corresponding to 6% of all adverse reactions reported in this catalogue. The most frequent groups of allergens inducing anaphylaxis reactions were drugs (83%), foods (7%) and Hymenoptera venom (3%). Of these allergens, the AAi is only indicated for food and venom allergy, corresponding in this study to a prevalence of patients with indications to AAi of 1.12/100,000 inhabitants (14).

The prevalence of patients at risk of anaphylaxis based on AAi acquisition and the annual cost inherent to these devices acquisition in Portugal are unknown. Our aim was to evaluate the frequency of AAi acquisition in Mainland Portugal during a period of 15 years (2003-2017), and to calculate the economic impact in terms of cost inherent in its acquisition for the patients and the National Health System (NHS). We also estimate the risk of anaphylaxis occurrence based on this data.

Materials and methods

The frequency distribution of AAi acquisition in Portugal between 2003 and 2017 was analyzed through data provided by INFARMED (National Authority of Medicine and Health Products I.P.). During this period, two brands of AAi were marketed with 0.15 mg/0.3 mL and 0.3 mg/0.3 mL dosages.

To adjust the dosage to weight and age, the studied population was divided into two age groups: group A – patients aged between 5 and 9 years for the 0.15mg dose, and group B – patients older than 10 years (≥ 10) for the 0.3 mg dose.

The doses relating to bodyweight recommendations are based on limited pharmacokinetic data in healthy volunteers. No pharmacokinetic or pharmacodynamics studies involving patients with anaphylaxis have been published. They are also based on consensus and standard practice. For children under 15 kg adrenaline autoinjectors are not usually recommended; the recommendation for children 15-30 kg is a 0.15 mg adrenaline autoinjector device and for children over 30 kg and adults an 0.3 mg adrenaline autoinjector device (2, 15). Considering that, according to the growth reference values of Portuguese children, a 5 years old child has an ideal weight of 18.4 kg and a 10 year old child 31.9 kg, we chose to use the range of 5-9 years and over 10 to define the age groups.

The prevalence/inhabitant calculation was performed using the National Statistics Institute database for Mainland Portugal resident population during this period, based on the 2011 census. The devices acquisition per geographic regions (North, Center, Lisbon, Alentejo and Algarve) according to the Nomenclature of Territorial Units for Statistical (NUTS 2013) was also calculated. Considering the price of the devices in 2017, the inherent cost to the patient and the NHS was evaluated.

Data analysis was performed using Microsoft® Excel 2016. Data were anonymized and patient confidentiality was guaranteed. The study protocol was approved by the Ethical Board of Centro Hospitalar Universitário de Lisboa Norte.

Results

We show in **figure 1** the distribution of the frequency of AAi acquisition.

In 2003 and 2017, the lowest and highest numbers of AAi units were acquired for both doses, respectively. There was a progressive increase in acquisition over the years for both dosages. For the 0.15 mg dose, there was with an average increase of 117 units per year, with the largest increase from 2010 to 2011, representing an increase of 389 units acquired. The average annual increase for 0.3 mg dose was 349 units per year with the most significant increase occurring from 2016 to 2017, representing an increase of 1368 units acquired (**figure 1A**).

The prevalence of acquisition per inhabitant in 2003 for the dose of 0.15 mg was 13 per 100,000 inhabitants, followed by a progressive increase in AAi dispensed, reaching a maximum of 376 per 100,000 inhabitants in 2017, which correspond to a total average of 144 units per 100,000 inhabitants acquired over these 15 years. For the 0.3 mg dose, in 2003 the prevalence was also the lowest, with a total of 3 units acquired per 100,000 inhabitants, and in 2017 the largest (58 per 100,000 inhabitants), corresponding to a total average of 21 units per 100,000 inhabitants acquired for the 0.3 mg formulation (**figure 1B**).

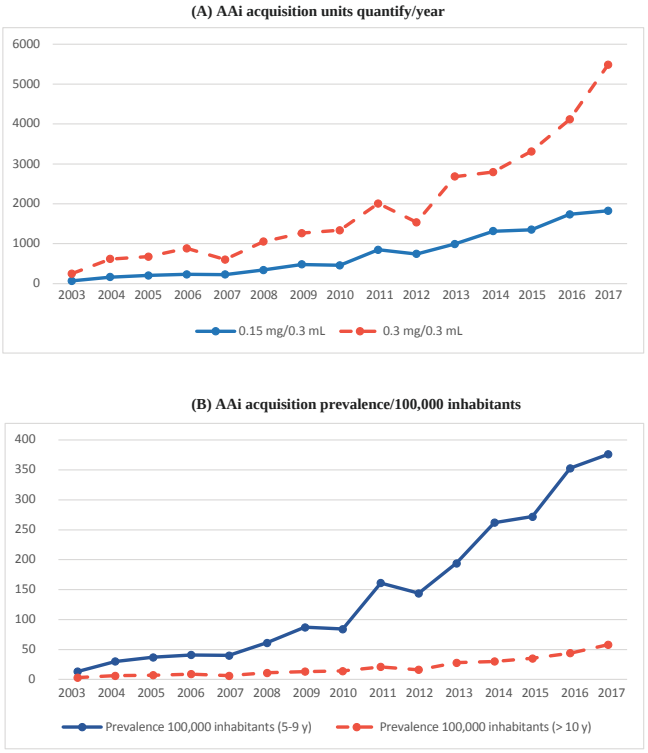
According to our data, based on the adrenaline acquisition over these 15 years, it is possible to predict a population-at-risk of anaphylaxis ratio of 0.165%. This corresponds to a dispensing average of 165 units of AAi per 100,000 inhabitants.

Regarding the cumulative value of devices purchased per year and region, Lisbon was the one with the most AAi devices purchased in both marketed doses: A: 40%, B: 43%, followed by the North with A: 32%, B: 25%, the Center region with A: 21%, B: 23%, Algarve with A: 4%, B: 5% and Alentejo with A: 3%, B: 4% (**figure 2**).

In the last year of the study, in terms of acquisition prevalence per 100,000 inhabitants in the different regions, Lisbon continued to be the one with the most units dispensed (84), followed by the Center region – 75, Algarve – 73, North – 71 and finally Alentejo – 33 (**table I**).

Also, in 2017, considering the two brands marketed in Portugal, the average cost per device has A: €55.81 and B: €54.29. With the NHS co-payment of 37% since 2009, cost per unit per user (A/B) was €35.16/€34.20 and for the NHS €20.65/€20.09, which corresponds to an annual cost of €64,202.71/€187,447.70 for users and €37,706.35/€110,1103.30 for the NHS. These data are summarized in **table II**.

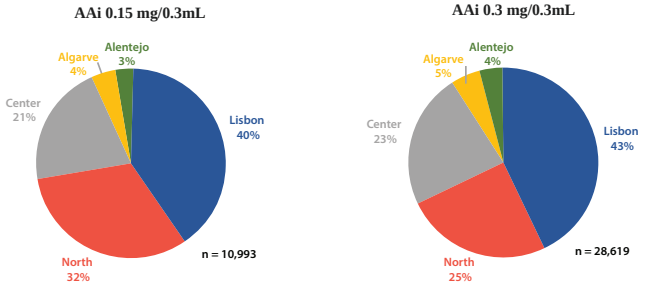
Figure 1 *- Adrenaline autoinjector acquisition in Mainland Portugal 2003-2017.*



Population A (5-9 years): 544,632 on 2003; 546,190 on 2004; 552,868 on 2005; 557,881 on 2006; 559,471 on 2007; 559,132 on 2008; 553,680 on 2009; 540,608 on 2010; 527,769 on 2011; 518,699 on 2012; 510,652 on 2013; 503,601 on 2014; 497,237 on 2015; 292,355 on 2016; 486,308 on 2017.

Population B (> 10 years): 9,350,364 on 2003; 9,375,678 on 2004; 9,376,306 on 2005; 9,417,926 on 2006; 9,446,232 on 2007; 9,471,501 on 2008; 9,496,226 on 2009; 9,526,009 on 2010; 9,535,039 on 2011; 9,510,688 on 2012; 9,474,419 on 2013; 9,440,820 on 2014; 9,417,757 on 2015; 9,460,792 on 2016; 9,387,089 on 2017. *Data from the National Statists Institute.

Figure 2 *- Adrenaline auto-injector acquisition distribution per geographic regions 2003-2017.*



Discussion

According to our results, in the last 15 years there was a large increase in AAi acquisition by 26 times for the pediatric patients and by 21 times for adolescents/adults. Other published studies also showed an increase, although of a lower magnitude. For example, Levy *et al.* (10) demonstrated an increase in AAi acquisition by the population of Israel between 1997 and 2004 of 59% in the pediatric formulation and 89% in that of adolescents and adults. The review of pharmaceutical data in Australia between 1998 and 2002 by Kemp (16), demonstrated an increase in AAi dispensing to pharmacies by 300% of the pediatric formulation and 193% of the adolescents and adult's formulation. In the United Kingdom, the increase in AAi prescription over 13 years (1991-2004) was 1200% (17).

The increase can be explained by the higher reports on food and venom anaphylaxis cases but also by the NHS co-payment that has been in existence since 2009, acquisition by the health professionals themselves such as private clinics, dentists and immunoallergologists, and better knowledge through health education training within medical professionals, and due to the short expiration date of the device.

There was a higher AAi acquisition rate per inhabitant in the younger group in relation to the adolescent/adult group, which is in accordance with other studies published (9-11, 18-20).

Simons *et al.* (9), using a pharmaceutical database, analyzed data from all formulations of epinephrine dispensed for 5 years in Manitoba (Canada), demonstrating an average dispensing rate of 1.44% in patients under 17 years and 1.22% in patients over 17 years of age. Taking into account the age distribution used to account for the AAi dosage and the number of people included in each group, over the 15 years, our study predicts an average dispensing ratio per 100,000 inhabitants of 0.144% for the 5 to 9 years group, and 0.021% for the group aged 10 years or more, comparatively much lower for both groups.

According to our data it is possible to predict a population-at-risk of recurrent anaphylaxis ratio of 0.165%. This corresponds to a dispensing average of 165 units of AAi per 100,000 inhabitants. This value is in the range of other studies published using the same methods (9,10). Levy *et al.* predicted an incidence of anaphylaxis of 0.083% (83 per 100,000) in the Israel population between 1997-2004 (10). The other study estimated the occurring anaphylaxis rate in the population of Manitoba, Canada (1995-2000) at 0.95% (954 per 100,000) (9). Our frequency is within the limits published on other studies but lower than this last study, allowing us to infer that in Portugal, as in many other countries, anaphylaxis is underdiagnosed and underreported. Besides that, anaphylaxis seems to be undertreated; even if it is properly diagnosed, some patients are not prescribed with AAi – *e.g.*, in the emergency department –, and even if it is prescribed,

Table I *- Adrenaline autoinjector acquisition distribution per geographic regions in 2017.*

Geographic Region	AAi 0.15 mg/0.3 ml*	AAi 0.3 mg/0.3 ml*	Mean population years/ region 2017**	Prevalence/region (A+B) 100,000 inhabitant 2017**
Alentejo	34	203	715,019	33
Algarve	65	261	440,543	73
Center	399	1309	2,237,640	75
Lisbon	738	2206	3,580,390	84
North	590	1452	2,827,514	71
Total	**1826**	**5481**	**9,801,106**	**336**

AAi: adrenaline autoinjector; *data from the National Health System (NHS)/National Authority of Medicine and Health Products I.P. (Infarmed); **data from the National Statistics Institute.

Table II *- Adrenaline auto-injector costs in 2017.*

Parameters	Adrenaline auto-injector dose	
	0.15 mg/0.3 mL	0.3 mg/0.3 mL
Average price/unit (€)	55.81	54.24
NHS co-payment (37%)/unit (€)	20.65	20.09
Cost per patient (€)	35.16	34.20
Cost for patients in 2017 (€)	64,202.71	187,447.70
Cost for NHS in 2017 (€)	37,706.35	110,113.30

NHS: National Health System.

they often do not acquire it, mostly because of the price or because they don't understand when or how to use it.

Regarding the distribution per geographic region, Lisbon contributed the highest percentage of devices purchased for both dosages, either in terms of the cumulative average value of devices purchased per year per region or per 100,000 inhabitants, while Alentejo consumed the least, substantiating that the consumption is proportional to the population density.

In our study, in both groups, there was a progressive increase in the prevalence of AAi acquisition, which also denotes an increase in the prevalence of patients at risk of anaphylaxis, reaching in 2017 a consumption of 434 units per 100,000 inhabitants that represents a 0.43% rate of population at risk of anaphylaxis in our country this year, closer to world values (21, 22).

Considering that this is an analysis of acquisition, this study showed a higher number of anaphylaxis cases per 100,000 inhabitants compared to another Portuguese study (14), which evaluates the registry of anaphylaxis cases in the CPARA (which only represents anaphylaxis reports done by an healthcare professional), implying that could have a underreport by health professionals, since a large number of cases in the CPARA correspond to drug allergy, in which case AAi prescription is not indicated. However, our data is not a direct estimate of the number of anaphylaxis cases. We use a "surrogate marker" of anaphylaxis cases to estimate the frequency of its occurrence. The crude (not per patient) AAi acquisition data may have 2 or 3 devices per year for the same anaphylaxis patient; that is a major difference between our data and CPARA.

Patients with a previous history of anaphylaxis should be studied in an Immunoallergology outpatient clinic to obtain a correct diagnosis and adequate therapeutic orientation in order to reduce the risk of future reactions. Although the guidelines promote the AAi device prescription (7), as well as referral to specialized consultation, this is often not verified. Some studies have shown that the percentage of patients observed in the emergency department with suspected anaphylaxis for whom AAi is prescribed varies between 16-63% (23-25) and that the percentage of referral to a specialized consultation ranged from 11-33% (26-28). Some factors that may contribute to these low rates are the high cost of the devices, waiting lists for the specialized consultation conditioning delays in the study or withdrawal by the patients themselves, and also the non-acquisition of AAi due to the expiration of the prescription.

In 2017 the AAi acquirement in Mainland Portugal reached the maximum of units acquired implying increased costs. The annual cost for the 0.15 mg AAi dose corresponds to about

€65,000.00 for pediatric patients, and for the NHS about €38,000.00 with an average cost per device of €35.16. Diwakar *et al*. in 2017 reported that the UK's current annual expenditure on AAi for children is approximately £7,000,000.00 with an average cost per unit of £25.80 (29).

For the adolescent and adult population, the annual cost in 2017 was about €190,000.00 for the patient and €111,000.00 for the NHS, with a cost per device of €34.20. Patel *et al*. (30) estimated that the AAi device acquisition correspond to an annual average cost of $20 million for devices purchased (cost/device $51).

The frequency of population-at-risk of anaphylaxis and its treatment, namely the acquisition of AAi, impose high costs. Despite the current 37% co-payment by the NHS for these devices, the cost is still high since annual renewal is necessary. Compared to the UK, an AAi is less expensive in the countries where the medium monthly income is higher.

Some limitations of our study are that although is divided per age groups, age range < 5 years is not included since the adrenaline dose must be adjusted for weight and at these ages is rarely prescribed. The etiology of anaphylaxis for which AAi was prescribed is not known, as the main cause of adult anaphylaxis is drug allergy, and in this case AAi device is not indicated. Also, the fact that is an acquisition and not a prescription study and the acquisition depend on the patients. Population division in urban and rural areas was not possible, which would be important due to the higher prevalence of anaphylaxis to Hymenoptera in rural areas. However, there was a higher rate of anaphylaxis in areas with higher population density as expected.

There has also been a greater education for health among professionals in the most diverse areas. This also allows a greater number to be aware of the need for prescribing adrenaline in some patients. Children are very common to carry more than one device, even if it is not mentioned in the guidelines (*e.g*., one at home, one at school, one at grandparents' home), this may leads to an overestimation of the prevalence of patients at risk using this methodology. We estimate the rate of patients at risk for anaphylaxis is based on acquired AAi but not the total anaphylaxis rate in Mainland Portugal.

Conclusions

In the last 15 years, there has been an increase in AAi acquisition for both the pediatric age and adolescents/adults. The significant rise in the number of prescriptions per year suggests an increase in the prevalence of patients at risk of anaphylaxis based on acquired AAi in Mainland Portugal reaching 0.165%.

The authors also justify the significant increase in AAi acquisition, not only due to the rise in anaphylaxis cases frequency, but also as a result of the greater knowledge through health education training in the population and medical professionals, as well as the AAi device co-payment by the NHS in recent year.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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Home administration of biological treatment in severe asthma in real-life experience: impact on asthma control and quality of life

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Key words

Biological treatment; severe asthma; home administration; self-administration.

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Impact statement

Home administration of biological treatment in severe asthma did not lead to any deterioration of asthma control or quality of life in real-life experience.

Summary

Introduction. *Several biological agents for the treatment of severe asthma have been approved for self-administration on an outpatient basis in the last years. However, data on the impact of home administration in outcomes such as asthma control and quality of life in real-life settings are sparse. Being this knowledge crucial for clinical practice, this study aimed to assess asthma control and quality of life in patients who transitioned from day hospital administration of biological therapy to home administration.* ***Methods.*** *A single-center prospective analysis of 33 patients treated with biologics for severe asthma, who switched from hospital to home treatment was performed. Asthma Control Test (ACT), Control of Allergic Rhinitis and Asthma Test (CARAT), Asthma Life Quality (ALQ) and the number of exacerbations were assessed 3 months before and 3 and 6 months after of home-use.* ***Results.*** *ACT and CARAT did not show statistical differences comparing to the baseline values (21.8 ± 2.7 and 23.8 ± 5.5) within 3 months (22.1 ± 2.4, p = 0.609; 23.2 ± 5.3, p = 0.572) or 6 months (23.4 ± 0.9, p = 0.553; 23.7 ± 6.2, p = 0.149) of home administration. Also, ALQ score did not show meaningful variations between baseline (9.5 ± 3.2) and after 3 months (11.2 ± 4.4, p = 0.275) and 6 months (10.3 ± 3.8, p = 0.209) of home-use. Regarding asthma exacerbations, we did not record a significant difference comparing to the baseline values of 3 months/patient exacerbations (0.2 ± 0.4) and after 3 months (0.2 ± 0.5, p = 0.786) or 6 months (0.2 ± 0.4, p = 1.000) of change in modality treatment. There were no cases of anaphylaxis or other serious adverse effects in those patients treated at home.* ***Conclusions.*** *Transition of day hospital administration of biologic treatment for severe asthma to home administration did not lead to any deterioration of asthma control or quality of life. Our results emphasized the efficacy and safety of home administration of biologic treatment and provide support on changing the paradigm of the administration of biological treatment in severe asthma.*

Introduction

Severe asthma affects approximately 5-10% of all asthmatic patients and it is characterized by an insufficient response to treatment with high doses of inhaled corticosteroids and a second controller (1, 2). In recent years, there has been a remarkable progress in the treatment of asthma due to a better understanding of its complex pathophysiology and the introduction of several biological drugs (3). Recently, four out of five biologics have been approved for patient self-administration at home in Europe. The advantages of self-administration of drugs have already been highlighted in the literature, as well as being clearly recognized in the treatment of rheumatoid arthritis and psoriasis. These studies stated the efficacy and safety of the treatments, besides the greater patient adherence to therapy (4-6). The out-of-hospital administration of biologic agents in severe asthma has been addressed by several studies that evidenced the added-value of this kind of administration (7-9). For instance, some studies demonstrated that patients can safely and effectively self-administer biological agents through proper training and no cases of anaphylaxis, suspected allergic reactions or other serious adverse effects related to biological treatment were reported. Also, patient satisfaction related to self-administration has been reported (10, 11). In the era of

Coronavirus disease 2019 (COVID-19), international respiratory societies (Global Initiative for Asthma, European Respiratory Society, British Thoracic Society and American Academy of Allergy, Asthma & Immunology) updated their guidelines favoring the practice of self-administration of biologics. This recommendation of self-administration of biologics at home was followed by some centers during lockdown periods that revealed to be a success (12, 13). Nevertheless, it is needed to collect more data to clearly state the advantages of patient self-administration of biological drugs at home. Therefore, the aim of this study was to assess asthma control and quality of life in patients who transitioned from day hospital biological therapy administration to home administration at the Pulmonology Department of Centro Hospitalar e Universitário de Coimbra.

Methods

Study design

The current work was a prospective observational real-life study performed on patients treated with biologics for severe asthma, who switched from hospital to home treatment, between May 2020 and July 2021. The study took place in severe asthma unit from Pulmonology Department of Centro Hospitalar e Universitário de Coimbra. The research was conducted in accordance with the ethical standards established in the Declaration of Helsinki and informed consent was obtained from all participants before enrolment in the study.

This study included 18-year-old patients or older with severe asthma who were receiving treatment with biologic agents for at least 6 months. We used a protocol to support the process of self-administration, which comprised: 1) a questionnaire to the patients aiming to evaluate their acceptance level of the home administration modality, the patient perception of the advantages and drawbacks of this kind of administration, as well as to give the opportunity to the patients expose their concerns; 2) the decision of the coordinator of severe asthma unit on the patient overall conditions to comply with the modality treatment; 3) a teaching/training period of self-administration in the day hospital by the nurses team. Furthermore, patients were asked what kind of support information they would prefer regarding self-administration (video, written information). Patients received two or three doses under supervision with training on how to self-administer (according to the ability of the patient), followed by home self-administration for the remainder of the follow-up time. Telephone calls were made by the nurse to check if the auto-administration was performed at the correct time and reinforce adherence to therapy.

Clinical monitoring and patient follow-up after home treatment initiation was performed at least twice: at 3/4 months and at 6 months of treatment. At each patient visit the following parameters were recorded and comparatively evaluated: asthma control, asthma-related quality of life, number of exacerbations and adverse effects. The determination of these parameters is described in detail in the next sections. The technique was reviewed by the nurse.

Study measurements

Asthma control

Symptom control was assessed using the Asthma Control Test (ACT) and the Control of Allergic Rhinitis and Asthma Test (CARAT) at baseline, at 3 months and at 6 months after home treatment initiation. Scores at 3 and 6 months were compared to baseline.

The ACT is a self-administered five-item tool for identifying patients with poorly controlled asthma with a score ranging from 5 to 25. A score of 20 to 25 means that the asthma is well controlled; a cut off score of ≤ 19 indicates poorly controlled asthma in which a score of 16 to 19 is considered partially controlled asthma; and < 16 indicates uncontrolled asthma (14). Individual ACT score changes of ≥ 3 were considered to be clinically meaningful (15). A validated Portuguese-language version of the ACT was used (16).

CARAT is a brief self-administered Portuguese questionnaire divided into two sections: the first part evaluates the symptoms of allergic rhinitis through four questions, in which a total > 8 means good control; and the second part evaluates the symptoms of asthma in six questions, with good control defined as values > 16. Asthma was considered controlled for CARAT global score above 24 (17, 18).

Asthma-related quality of life

The quality of life was measured by Asthma Life Quality (ALQ) test, a self-administered questionnaire that comprises 20 questions in yes/no answer format. It addressed six dimensions of asthma's impact in patients' lives: activity and sleep, symptoms, triggers, unscheduled health care use, medication and psychological. All questions had equal weight and the total ALQ score is calculated as the sum of all positive (yes) responses, ranging from 0 to 20 (19). Lower scores reflect greater quality of life impairment. The Portuguese version of the ALQ was previously translated, adapted and validated (20).

Exacerbations

An exacerbation was defined as worsening of asthma symptoms, requiring the administration of oral corticosteroids (OCS) for at least 3 days or if the patient had visited an emergency department or was hospitalized. Exacerbation rates in the 3 months before home transition were compared to the number of exacerbations at 3 and 6 months following home treatment.

Adverse events

Safety was assessed by the collection and description of drug-related adverse events (AEs) during the study. The investigators determined the relationship of the AE to the different biologic agents.

Statistical analysis

Data at baseline were expressed as mean ± standard deviation (SD) for continuous variables and in terms of number and percent (n, %) for categorical variables. The normality of data distribution was assessed using Kolmogorov-Smirnov test. The statistical analysis used to assess the results obtained after transition to home administration was the Paired samples t-test for paired samples. A statistical significance level of 0.05 was used. Statistical analysis was performed by using the SPSS 25.0 software.

Results (figure 1)

Baseline patients' characteristics

A total of 33 patients from a population of 57 patients (57.9%) with severe asthma receiving biologic treatment were selected by the assistant physician to home administration and were enrolled in this study. Two patients were excluded because they did not adhere to the therapy on home modality and, subsequently, they were transferred back to the day hospital administration.

Clinical characteristics of the study population are shown in **table I**. The mean age of the population was 43.6 ± 16.3 years with a predominance of women (69.7%).

Before home administration, mean ACT scores were 21.8 ± 2.7 points. Mean CARAT score in the upper airways was 8.6 ± 3.1 and in the lower airways 15.1 ± 3.5, with mean of total CARAT score of 23.8 ± 5.5. The initial mean value for ALQ was 9.46 ± 3.2. The mean number of exacerbations in the past 3 months varied between 0 and 1, with most of the patients (84.8%) having none.

Concerning the biologic treatment administrated, 51.5% of patients were treated with omalizumab, 24.2% with mepolizumab, 15.2% with benralizumab and 9.1% with dupilumab. The mean duration of the biologic treatment as an add-on maintenance therapy was 3.0 ± 2.9 years. No patients were on OCS.

Symptom control

Asthma control based on the ACT scores did not show statistical differences comparing to the baseline (21.8 ± 2.7) at both 3 months (22.1 ± 2.4, p = 0.609) and 6 months of home treatment (23.4 ± 0.9, p = 0.553). No patients showed a decrease of 3 or more points in ACT score at 3 and 6 months of home modality, representing a stability in asthma control. Regarding CARAT total score, there were no significant variation comparing the baseline (23.8 ± 5.5) with the mean score at 3 months (23.2 ± 5.3, p = 0.572) and at 6 months (23.7 ± 6.2, p = 0.149). Similarly, baseline CARAT score of the upper airways (8.7 ± 2.8) and of the lower airways (14.3 ± 4.2) did not show significant difference after 3 months of home-use (8.7 ± 3.8, p = 0.876 and 15.1 ± 2.6, p = 0.145) and also after 6 months (8.8 ± 3.7, p = 0.855 and 14.6 ± 2.9, p = 0.118).

Table I *- Baseline patients' characteristics enrolled in this study.*

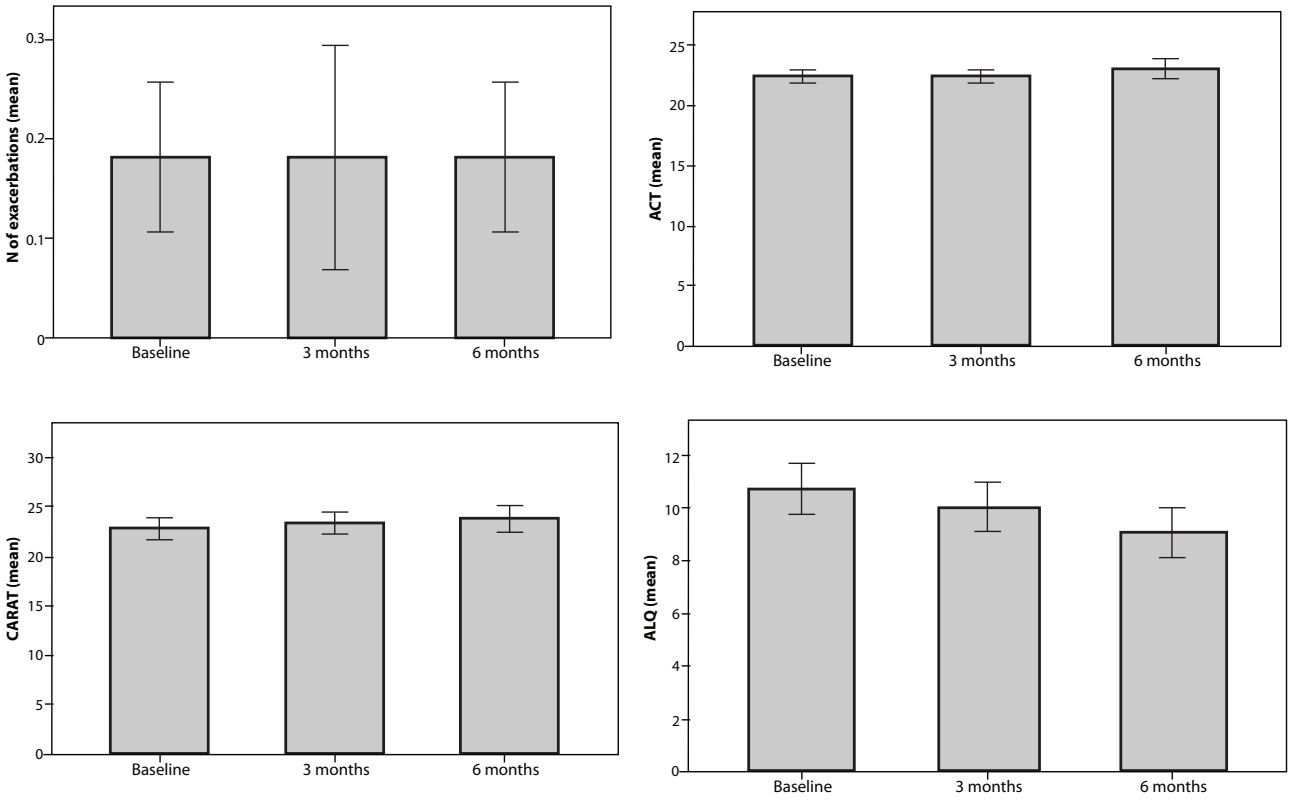
Variable	n = 33
Gender	
Female: n (%)	23 (69.7%)
Male: n (%)	10 (30.3%)
Age (years)	
Mean ± SD	43.6 ± 16.3
Range	19-70
Age group (years): n (%)	
18-34	9 (27.3%)
35-64	19 (57.6%)
≥ 65	5 (15.1%)
BMI (Kg/m^2): mean (SD)	27.9 ± 6.6
ACT	
Total score: mean (SD)	21.8 ± 2.7
Well-controlled asthma (score ≥ 20): n (%)	22 (66.6%)
Partly controlled asthma (score 19-16): n (%)	13 (39.4%)
Uncontrolled asthma (score < 16): n (%)	0 (0%)
CARAT	
Total score: mean ± SD	23.8 ± 5.5
Control asthma (score > 24): n (%)	18 (54.5%)
Upper airway: mean ± SD	8.6 ± 3.1
Control upper airway (score > 8): n (%)	17 (51.5%)
Lower airway: mean ± SD	15.1 ± 3.5
Control lower airway (score > 16): n (%)	18 (54.5%)
Exacerbations in the previous 3 months	
Median (range)	0 (0-1)
0: n (%)	28 (84.8%)
1: n (%)	5 (15.2%)
ALQ	
Total score: mean ± SD	9.46 ± 3.2
Spirometry	
Pre-BD FEV1 (ml): mean ± SD	2.73 ± 0.8
Pre-BD FEV1 (%): mean ± SD	88.0 ± 18.6
FEV1/FVC (%): mean ± SD	74.2 ± 13.6
Post-BD FEV1 (ml): mean ± SD	2.79 ± 0.8
Post-BD FEV1 (%): mean ± SD	91.1 ± 17.1
Biologic treatment: n (%)	
Omalizumab	17 (51.4%)
Mepolizumab	8 (24.2%)
Benralizumab	5 (15.2%)
Dupilumab	3 (9.1%)

ACT: Asthma Control Test; ALQ: Asthma Life Quality; BD: bronchodilator; BMI: body mass index; CARAT: Control of Allergic Rhinitis and Asthma Test; FEV1: forced expiratory volume at 1st second; FVC: forced vital capacity.

Exacerbations

There was no significant difference in exacerbation rate comparing to the baseline value of 3 months/patient exacerbations (0.2 ± 0.4) at both 3 months (0.2 ± 0.5, p = 0.786) and 6 months of home treatment (0.2 ± 0.4, p = 1.000).

Figure 1 *- Evolution of the number of exacerbations and ACT, CARAT and ALQ scores from baseline to 3 and 6 months after transition to home administration of biologic treatment.*



ACT: Asthma Control Test; ALQ: Asthma Life Quality; CARAT: Control of Allergic Rhinitis and Asthma Test.

Quality of life

There were no meaningful variations in ALQ score after 3 months (9.5 ± 3.2 *vs* 11.2 ± 4.4, p = 0.275) or 6 months (10.3 ± 3.8, p = 0.209) of home treatment.

Adverse events

There were no cases of anaphylaxis or other serious adverse effects in the patients treated at home. The related adverse effects were headaches (two cases), pain at the injection site (one case), hair loss (one case) and arthralgia (one case). Two patients discontinued home biologic treatment for non-compliance to the drug and none of the patients discontinued because of adverse effects. There was no difference in adverse effect frequency/severity seen between the home-treated and hospital-treated patients.

Discussion

In this study, we report a real-world experience of use of different biologic agents in home administration in patients with severe asthma. We observed that transition to home administration had no negative impact on adherence and did not lead to any deterioration of asthma control or quality of life, as highlighted by the absence of modification in the ACT, CARAT and AQLQ scores. Additionally, we did not record an increase in the number of reported exacerbations.

Adherence to home treatment in our study appears to be excellent, with only two patients discontinuing this modality of treatment for non-compliance reasons. Overall results of this study indicate that almost all patients were adequately trained to administer the treatment at home and that communication with the patient and confirmation of administration is crucial to enhance adherence to therapy. Additionally, understanding the individual preferences and concerns regarding self-administration at home may also improve adherence to therapy and well-being of the patient (21). These results corroborated previous results (12, 22) that reported self-administration can be a useful tool to maintain adherence to biological therapies. This is notable as it is well recognized that adherence to asthma inhaled

therapy tends to be very poor, with the reported rates of nonadherence ranging from 30 to 70% (23).

The asthma control assessed with validated tools did not show any deterioration when the treatment was switched to home administration. This finding corroborated the results of other previous studies. In 2007, Liebhaber *et al.* (9) reported their experience on 25 patients with allergic asthma undergoing long-term at home treatment with omalizumab demonstrating for the first time that patients can effectively self-administer omalizumab at home. Although efficacy measures were not a primary endpoint of the study of Liebhaber *et al.*, patients showed clinical improvement in symptoms of asthma. More recently, the GREGALE study assessed the functionality of an accessorized pre-filled syringe to administer a fixed dose of benralizumab both in a healthcare setting and at home, in patients with severe uncontrolled asthma (8). This study showed an improvement in asthma control as represented by the decreased in the mean Asthma Control Questionnaire 6 score compared with the baseline values. Several studies performed during COVID-19 lockdown also show that self-administration of biologics at home did not induce any significant change related to severe asthma control or exacerbations rate (12, 13).

In this present study, we did not record a worsen in patient's perception quality of life, since there was no statistically significant difference in the ALQ score in patients who transitioned to home administration. Although in our study we did not directly explore the patient satisfaction with home administration, the medication and psychological perception assessed by ALQ allowed us to infer about it.

In our study, no anaphylaxis was reported with biologic administration in an at-home setting and the safety profile was comparable to that observed in other studies (7-9, 24-27). This is an encouraging aspect for the at-home self-administration of biological drugs for severe asthma. Nevertheless, a longer duration study will be important to assess long-term safety of biologic administration in an at-home setting.

The main findings of our study are quite encouraging about home administration of biological treatment in severe asthma; however, we are aware of some methodological limitations. First, our study was based in a heterogenous asthmatic population under the treatment of four different biologic agents, but we believe that these biases had a low impact in our conclusions as we proved the absence of deterioration of asthma control in every single patient. Second, the sample population size was small, but this was the representation of a real-life experience in a dedicated single-center. To increase the population size, more centers should be involved. Third, even though this study was conducted in a real-life setting with a longer follow-up period (6 months), more extended follow-up times would be necessary to establish the efficacy and safety for longer observation times.

Apart from its limitations, this study presented relevant strengths. To the best of our knowledge, this is the first real-life study of home administration of different biologic agents in treatment of severe asthma addressing the benefit of biological treatment in terms of important clinical aspects, such as symptom control and quality of life, that are the most perceived results by the patient.

Conclusions

In conclusion, our real-life experience supported the efficacy and safety of home administration of biologics agents in the treatment of severe asthma. Larger home therapy studies are needed to provide the evidence necessary to adequately reinforce the efficacy and safety of home administration of biologic treatment in severe asthma and therefore change the paradigm of the administration of biological treatment in severe asthma.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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Hypersensitivity reactions to COVID-19 vaccines: a case of eosinophilic pneumonia following Sinovac/CoronaVac vaccination

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Key words

COVID-19; COVID-19 vaccines; SARS-CoV-2; Sinovac/CoronaVac; eosinophiles; eosinophilic pneumonia; rash.

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Summary

Hypersensitivity reactions have been reported with COVID-19 vaccines. Acute eosinophilic pneumonia has not been reported yet after Sinovac/CoronaVac vaccine. A 73-year-old woman presented with maculopapular rash, cough and dyspnea following Sinovac/CoronaVac injection. The complete blood count (CBC) indicated eosinophilia, and further evaluation of the eosinophilia with CT and bronchoscopy confirmed a diagnosis of acute eosinophilic pneumonia. After methylprednisolone therapy, her rash resolved with marked improvement of the dyspnea. She is still on treatment and on the follow-up period, we plan to continue steroid treatment at least 3 months.

Impact statement

Aluminum adjuvants in vaccines may cause eosinophilic inflammation in the lungs. This immunologic reaction seems to be reversible. Th2-mediated eosinophilic immune responses is decreasing with steroid treatment and do not relapse over time.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is first detected in Wuhan, China, in December 2019, and then defined as a novel coronavirus which caused coronavirus disease 2019 worldwide named as "COVID-19 pandemic". A total of 163,312,429 confirmed cases of COVID-19, including 3,386,825 virus deaths have been reported worldwide as of 18th May 2021 (1). Vaccination is the most effective strategy to control the pandemic and COVID-19 vaccines were an urgent need for this pandemic. The first mass vaccination program started in early December 2020 and Pfizer/BioNTech BNT162B2, Moderna mRNA-1273 and AstraZeneca recombinant adenoviral ChAdOx1-S became first approved COVID-19 vaccines in the United Kingdom (U.K.) on 30th December 2020 (2). Pfizer/BioNTech BNT162B2 vaccine was listed for WHO Emergency Use Listing (EUL) on 31st December 2020. The SII/Covishield and AstraZeneca recombinant adenoviral ChAdOx1-S were given EUL on 16th February. The Janssen/Ad26.COV2.S, the Moderna mRNA-1273 and Thee Sinopharm COVID-19 vaccine was listed for EUL on 12th March 2021, 30th April 2021 and 7th May 2021, respectively (1).

COVID-19 vaccines are now available in many European countries, the United States (U.S.A.), and worldwide. As of 17th May 2021, a total of 1,407,945,776 vaccine doses have been administered

worldwide (1). Soon after global use of COVID-19 vaccines, severe allergic hypersensitivity reactions to mRNA-based vaccines were reported (2). For example, 11.1 cases of allergic reactions including anaphylaxis occurred per 1 million doses of the Pfizer/BioNTech BNT162B2 COVID-19 vaccination (3) and of 64,900 employees who received their first dose of a COVID-19 vaccine including Pfizer/BioNTech BNT162B2 and Moderna mRNA-1273 vaccines, acute allergic reactions were reported more frequently with the Moderna vaccine compared with Pfizer-BioNTech (4). For the Pfizer/BioNTech BNT162B2 COVID-19 vaccine, 71% of allergic reactions occurred within 15 min of vaccination (3). While there are no added adjuvants or preservatives in mRNA based novel COVID-19 vaccines, different stabilizers including polyethylene glycol (PEG), polysorbates, tromethamine/trometamol were found to be potential to elicit systemic allergic hypersensitivity reactions (5).

Sinovac/CoronaVac COVID-19 vaccine is a 2-dose β-propiolactone-inactivated, aluminum hydroxide-adjuvanted COVID-19 vaccine authorized by the China National Medical Products Administration on 6th February 2021 (6). Phase 3 trial in Brazil including 8,840 participants who received any dose/schedule of Sinovac product reported only mild or moderate adverse events (AE) which were most commonly pain at the injection site, headache, fatigue, and myalgia. There were few allergic reactions, and all were Grade 1 or 2 (6). 260 million doses of Sinovac/CoronaVac have been distributed to the public domestic and overseas markets for use in adults ≥ 18 years (6), and COVID-19 vaccination program has been started by Sinovac/CoronaVac and Pfizer/BioNTech BNT162B2 vaccines in Turkey on 14th January and 2nd April, respectively. As of 19th May 2021 total 26,869,851 doses COVID-19 vaccine including mostly Sinovac and fewer Pfizer/BioNTech BNT162B2 vaccines has been administered to healthcare workers and elderly population (7). In Turkey Phase 3 Sinovac/CoronaVac study including 13,000 healthy participants with the age of 18-59 years, severe adverse events have not been reported (6). However, COVID-19 vaccinations including m-RNA based vaccines and Sinovac/CoronaVac seems to be associated with acute allergic reactions. Even though anaphylaxis is rare, the other hypersensitivity reactions such as acute eosinophilic pneumonia may be associated with COVID-19 vaccinations. To the best of our knowledge acute eosinophilic pneumonia, rash and dermatitis has not been reported yet after Sinovac/CoronaVac vaccination. This paper therefore aims to provide a concise review of the diagnosis and management of vaccine related acute eosinophilic pneumonia and maculopapular rash through a case presentation.

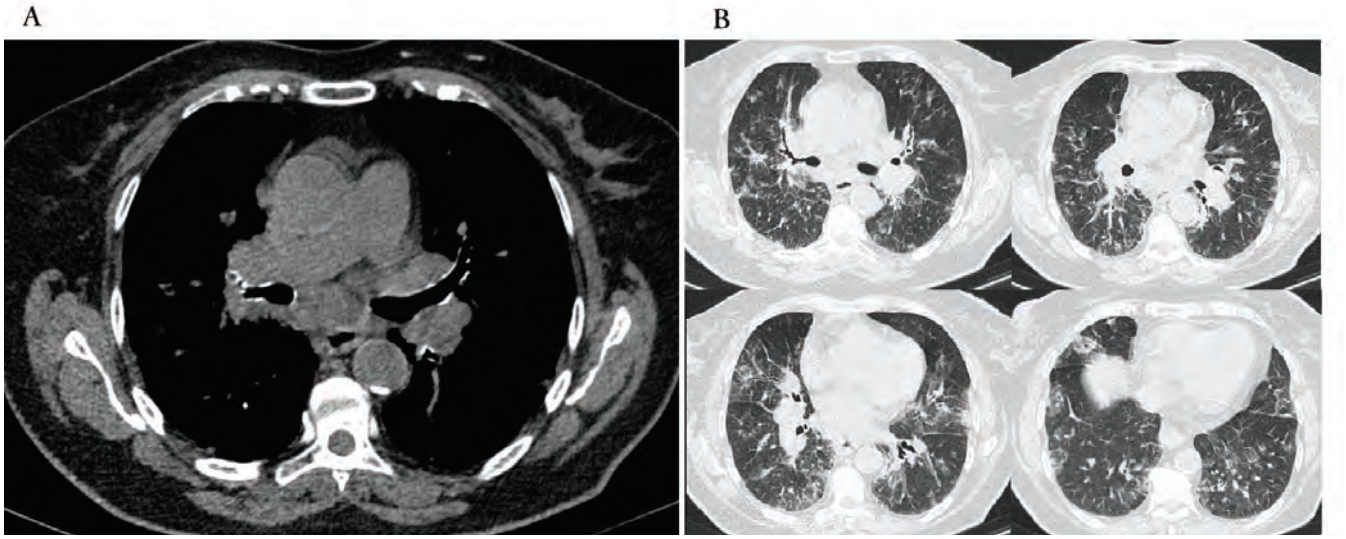
Case presentation

We report a 73-year-old woman who presented to our pulmonology and allergy clinic with maculopapular rash, cough and dyspnea after Sinovac/CoronaVac vaccination. Her cough started after first dose of the vaccine, and it was an isolated symptom and then manifested as maculopapular rash and dyspnea after 4th day of second dose of the vaccine. There was one month period between first and second dose of the vaccine. Antihistamines were not effective for her rash. The patient denied any allergy, history of allergic disease such as asthma or allergic rhinitis, newly started medication, herbal product use and smoking. She did not report any constitutional symptoms including weight loss, fever, chronic pain, fatigue, arthralgia, or night sweats. She had hypertension and diabetes history. Her vital signs were stable on presentation (**table I**). The CBC results indicated eosinophilia (eosinophile count = 600 k/μl). Further evaluation of the eosinophilia with CT scan could not exclude COVID-19 pneumonia (**figure 1**). Because she had dyspnea and there were diffuse ground glass densities, consolidation and linear densities in all segments of both lungs. While the SARS-CoV-2 PCR were negative and her anti SARS-CoV-2 anti-spike antibody level were positive at the effective level, favipiravir treatment was started. During the evaluation period, she had another negative PCR test for SARS-CoV-2. After third day of favipiravir treatment the oxygen saturation was 87% at room level and oxygen treatment were started. Blood eosinophile count has increased to 2300 k/μl. Fiberoptic bronchoscopy was performed and specimens of bronchoalveolar lavage (BAL) fluid obtained from right middle lobe. Multiple biopsies were obtained from the right lower lobe basal segments. Transbronchial needle aspiration (TBNA) was performed from 7 (right upper hilar) and 11 (left hilar) node stations by using EBUS. Skin biopsy was also obtained. The findings of BAL were as follows: macrophage 42% (normal > 90%), lymphocytes 11% (normal < 5%), neutrophils 11% (normal < 5%), and eosinophils 36% (normal < 1%). Eosinophile infiltration also detected in lung tissues (**figure 2**). There was no granuloma, malignant tumor or eosinophile infiltration in lymph nodes. Skin biopsy revealed oedema of the superficial dermis and a dense infiltrate of lymphocytes which was found to be associated with drug induced (vaccine) dermatitis. 1 mg/kg methylprednisolone therapy was started. After seven days of this treatment, her rash resolved with marked improvement of the dyspnea. Thereafter, the patient was continued on treatment with oral methylprednisolone (40 mg/day), the dose was planned gradually to be tapered after a period of 4 weeks. A follow-up chest X-ray revealed marked improvement and total Ig E decreased from 9662 IU/ml to 2000 IU/ml. Eosinophile levels was detected at the normal range. She is still on treatment and on the follow-up period we plan to continue steroid treatment at least 3 months.

Discussion

Eosinophilic pneumonia adverse reaction (AEs) after vaccination has rarely been reported. Only two cases have been reported following influenza and pneumococcal vaccination until today. To the best of our knowledge, this is the first report of acute eosinophilic pneumonia and maculopapular rash developed after Sinovac/CoronaVac vaccination. Based on 35.8 million doses distributed in Chine, 49 serious AEs reported, including anaphylaxis,

Figure 1 *- CT results.*



(A) Enlarged precarinal, subcarinal, pretracheal and paratracheal multiple lymph nodes were observed in the anterior mediastinum. The largest one was measured as 22 × 15 mm in the subcarinal area. **(B)** Diffuse ground glass densities, consolidation and linear densities in all segments of both lungs.

Table I *- Initial work-up of a case.*

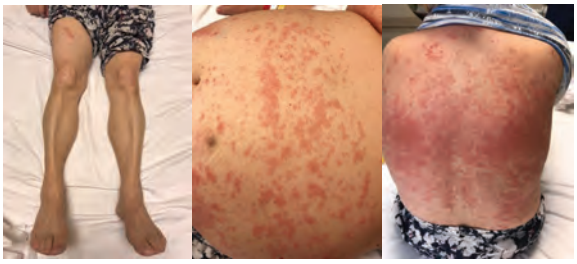
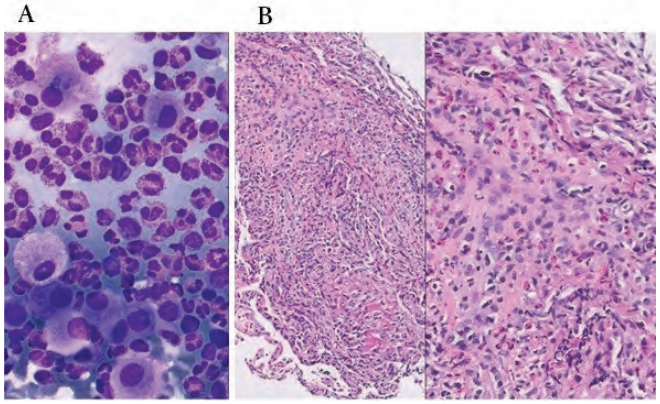
Physical examination	**Laboratory values**
Respiration: clear on auscultation bilaterally, no wheezes or crackles	CBC: Eosinophilia
Oxygen saturation: 92% at room air	WBC: 14,280 k/µl
	Lymphocyte count: 1,300 k/µl
	Eosinophile count: 600 k/µl (HIGH)
	CRP: 27.4 mg/l-D-Dimer: 3,170 µg/l
	Negative PCR test for SARS-CoV-2
	Anti SARS-CoV-2 antibody Ig G (anti-spike): positive
	IgE: 9,662 IU/ml
	LDH: 301 U/l
	Ferritin. 471 ng/ml
	IL-6: 5.3 pg/ml
Cardiovascular: clear S1 and S2, no extra sound	
No organomegaly (splenomegaly, hepatomegaly, lymphadenopathy)	
Generalized maculopapular rash	

Figure 2 *- Eosinophilic infiltration in the lung tissues and BAL.*



(A) Eosinophiles in the BAL. **(B)** Eosinophiles in the lung tissue.

Henoch-Schonlein purpura, laryngeal oedema, demyelination, cerebral hemorrhage (6). Based on 17 million doses distributed in Brazil/Indonesia, 162 serious AEs reported, including fever, dyspnea, death, and headache (6). Based on 3.7 million doses distributed in Chile, 90 serious AEs reported including anaphylaxis with the rate of 1.7/100,000 doses (6). While there were gaps in the detection of rare adverse events especially in older adults, there were no reported acute eosinophilic pneumonia and maculopapular rash after Sinovac/CoronaVac vaccination.

Sinovac/CoronaVac COVID-19 vaccine is an aluminum hydroxide-adjuvanted COVID-19 vaccine (6). Aluminum-containing compounds, primarily aluminum hydroxide (AH), have been widely used as adjuvants in the number of other vaccines such as hepatitis A, hepatitis B, diphtheria-tetanus-containing vaccines, *Haemophilus influenzae* type b, and pneumococcal vaccines (8). Immunization with aluminum adjuvants induces a Th2 type cell mediated immune response which plays an active role in development and differentiation of eosinophiles after the release of several cytokines including interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-5 (IL-5) (8). Aluminum adjuvant-containing vaccines do not activate Treg cells to control strong Th2-mediated immune responses (8). Therefore, aluminum adjuvants can induce the production of eosinophils and eosinophilia which may cause eosinophilic pneumonia or dermatitis after vaccination.

In the literature, two cases of eosinophilic pneumonia have been reported following influenza and pneumococcal vaccination. First case is 86-year-old Thai man with severe COPD presented with eosinophilic pneumonia after seven days of inactivated influenza vaccine (Vaxigrip, Sanofi Pasteur) injection (9). Second case is A 68-year-old Japanese woman presented with eosinophilic pneumonia which developed two days after she received her second vaccination with PPV23 (Pneumovax® NP) (10). Our patient was a 73-year-old woman who presented with maculopapular rash, cough and dyspnea after 4th day of second dose of the Sinovac/CoronaVac vaccine. Seasonal influenza (except Fluad) and PPV23 are adjuvant-free vaccines suggesting that the vaccination-associated eosinophilia in previous cases were not caused by aluminum adjuvants. In these patients without any adjuvants another pathway may lead to eosinophilia. However, in our case hypersensitivity syndrome associated with eosinophilic infiltration of the lung tissue could be related with aluminum adjuvants. Older age and repeated vaccine injections may increase the risk of hypersensitivity reactions and COVID-19 vaccines may also be more prone to allergic or hypersensitivity reactions. However, in cases of drug-induced eosinophilic pneumonia reported in the literature, skin eruption is never reported which is an important part of the clinical picture of our case. Skin rash was suggestive of DRESS (The Drug Reaction with Eosinophilia and Systemic Symptom) and features of the case were a delayed onset. However, the diagnosis of DRESS is challenging because the pattern of cutaneous eruption and the types of organs involved are various. In our case, organ involvement including kidney, hearth and liver, lymphadenopathy and fever was not detected. There was a lung involvement manifested as an eosinophilic pneumonia. We did not do a patch test with diluted vaccine which could better clarify the pathogenesis of the disease. It was our limitation. We used the RegiSCAR's scoring system which was published to classify the cases with DRESS reported in the literature (9) and our case had less likely DRESS when we used this scoring system. A clinical framework is given in **table II**.

This immunologic reaction seems to be reversible and previous case results indicate that Th2-mediated immune responses is decreasing with steroid treatment and do not relapse over time. However, there may be a relation between repeated aluminum adjuvants exposure and acute eosinophilic pneumonia frequency. A proven diagnosis of hypersensitivity to a vaccine component could be difficult. However, aluminum-containing vaccines such as Prevenar 13 should be avoided in patients who had a history of hypersensitivity reactions to any aluminum-containing vaccines. Based on the experience with other case reports, patients whose symptoms fully resolve after steroid treatment should be under treatment at least 3 months and should be followed up at least one year for the relapse (10, 11). Systemic corticosteroids also have been accepted as the gold standard treatment for clinical symptoms of DRESS too. Systemic corticosteroids are recommended to be tapered over 6 and 8 weeks to prevent the relapse of various symptoms of this syndrome and to be administered for 2 and 3 months (12).

Conclusions

In conclusion, diagnosing and treating patients who had hypersensitivity reactions after COVID-19 vaccines is challenging and there are still unanswered questions about the long-term adverse effects of the COVID-19 vaccines. Adjuvants and stabilizers such as polyethylene glycol (PEG) and aluminum seems to lead to allergic and hypersensitivity reactions. Pandemic is urgent and

Table II *- A clinical framework.*

Diagnostic criteria for DRESS by the RegiSCAR (12)	**Our case**	**Scoring system for classifying DRESS cases as definite, probable, possible, or no case, from Kardaun *et al.* (9)**
Acute rash	(+)	1
Reaction suspected drug-related	(+)	
Hospitalization	(+)	
Fever (> 38 °C)	None	-1
Laboratory abnormalities (at least 1 present) a) Lymphocyte above or below normal; b) Low platelet; c) Eosinophilia	(-) a) Lymphocyte count: 1,300 k/µl; b) Platelet count: 361,000 k/µl; c) Eosinophile count: 600 k/µl *Eosinophile count less than 700 k/k/µl is accepted as negative eosinophilia	0
Involvement of > 1 internal organ	Lung involvement	1
Enlarged lymph nodes > 2 sites	None	0
The first 3 criteria are necessary for diagnosis, and the presence of 3 out of the other 4	Final Score: 1	Final score < 2: no case Final score 2-3: possible case Final score 4-5: probable case Final score > 5: definite case

we need to continue vaccination. In the upcoming years, more data will become available to assess the incidence of different and rare hypersensitivity reactions related with various types of COVID-19 vaccines and then we may have a new perspective about the risk factors link with vaccine hypersensitivity reactions.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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Eosinophilic pneumonia following Sinovac/CoronaVac vaccination

Comment on "Hypersensitivity reactions to COVID-19 vaccines: a case of eosinophilic pneumonia following Sinovac/CoronaVac vaccination"

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Key words
Eosinophilic pneumonia; COVID-19; vaccination.

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Doi
N/A.

We would like to share ideas on the publication "Hypersensitivity reactions to COVID-19 vaccines: a case of eosinophilic pneumonia following Sinovac/CoronaVac vaccination" (1). Ozturk *et al*. reported a case and mentioned that "*Acute eosinophilic pneumonia has not been reported yet after Sinovac/CoronaVac vaccine*" (1). We agree that this patient had eosinophilic pneumonia. This might or might not be an adverse reaction to COVID-19 vaccine. Since there is no data on pre-vaccination health/immune status and there is also no complete investigation on other concurrent medical problem, it is still difficult to conclude that the problem is associated with vaccination. For example, the patient might have previous asymptomatic parasitic infestation that can concomitantly cause the lung hypersensitivity problem (2). Hence, it is necessary to investigate and rule out possible concomitant parasitic infestation, which is not an uncommon clinical problem.
The work did not require ethical approval or consent to participate.

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The authors declare that they have no conflict of interests.

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SARS-CoV-2 pandemics and RSV off-season outbreaks

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Key words

SARS-CoV-2; RSV; outbreaks; pandemic; seasonality.

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To the Editor,

the emergence of SARS-CoV-2 pandemics triggered a worldwide-scale implementation of measures that seriously impacted the circulation of respiratory syncytial virus (RSV), as demonstrated inter-seasonal RSV epidemics in some southern hemisphere countries and late off-season outbreaks in several European countries and USA.
In a multi-country longitudinal observation study, involving 18 countries that enforced non-pharmaceutical interventions (NPIs) to reduce the spread of SARS-CoV-2, You *et al.* found that all countries experienced delayed RSV onset and, based on data available by September 2021, in 61% there was a RSV rebound delayed by a range of 5 to 54 weeks (1).
The first example of shift in RSV seasonality was reported in Australia, in late 2020 and early 2021, with an off-season widespread of RSV infections after relaxing of COVID-19 restrictions, with unprecedented outbreaks and hospitalizations for RSV bronchiolitis, after being almost absent during 2020 winter (2, 3). Type A clades became dominant and responsible for the outbreaks in widely separated areas of Australia (4). The age distribution was also atypical with a higher-than-average number of RSV infections in older infants (3, 5).
Those findings were also reported in other southern hemisphere countries and raised the initial alert in the remaining globe, as mitigation measures for control of SARS-CoV-2 also impact dramatically the circulation of most respiratory viruses and may result in unusual seasonality and severe outbreaks of respiratory pathogens.
The reality observed in the northern hemisphere also began with marked reductions in RSV activity in early 2020. However, the so-called "first wave" of SARS-CoV-2, in March 2020, overlapped with the end of 2019-2020 bronchiolitis season. This resulted in a slight delay in the expected end of the epidemic bronchiolitis season, which explains why the reduced numbers of RSV activity were not as significant as those seen in southern countries.
When Stera *et al.* compared the 2019-2020 and 2020-2021 bronchiolitis seasons at a pediatric department in Italy, one of the world's first and largest clusters of SARS-CoV-2, discovered dramatic reductions in attendance and no hospitalizations for bronchiolitis during the epidemic season of 2020-2021 (6). Similar results were also found in Argentina, France, Belgium, and Japan at the beginning of the SARS-CoV-2 pandemic (7, 8).
That drop was correlated and coincident with pandemic-driven prophylactic NPIs as social distance, hand hygiene and face masks, together with lockdown, resulting in lower circulation of RSV and other airborne infectious agents (9).
Like southern countries in the late 2020, some European countries and US southern states have reported out-of-season spikes in RSV activity in early-mid 2021, mainly after April.
What is behind that surge? It's probably due to the easing of COVID-19 restrictions, as more people got vaccinated: the masks and social distances became optional in many countries, the schools opened, and the gathering promoted the rise in RSV cases.
Also, the lack of herd immunity to the disease due to lockdown that prevented contact with those viruses, thus reducing adaptive immune response, made children more susceptible to infection.
Another explanation could be the higher susceptibility due to the immunocompromised lungs of previously infected COVID-19 patients (1, 9).

The reduction of viral interference, a mechanism by which the replication of respiratory viruses can be inhibited by SARS-CoV-2 infection, could also explain the rising numbers (5, 10, 11).

There is uncertainty about the RSV and other respiratory virus behavior and how long it will take to resume; extrapolation from other pandemics may be important - the impact on respiratory virus circulation of 2009 H1N1, for example, persisted for many years (11).

Most works reporting the first off-season RSV epidemics raised the concern that in countries emerging from pandemic restrictions, the reintroduction of respiratory viruses within pediatric communities that have never contacted them, could result in uncontrolled transmission, probably at unusual times and with a higher magnitude and severity of cases (2-5).

Fortunately, several studies in different countries, while confirming earlier peaks and higher numbers of RSV than the usual pre-pandemic seasons, didn't confirm the higher severity of bronchiolitis episodes (5, 8).

More recent works, including the last cold season 2021-2022, confirm the epidemiological changes described in the previous season and the need to pay attention to the consequences of lack of immunity, in particular to those viruses that didn't resurge or did it weakly (5, 7).

Kume Y *et al.* examined changes in the detection rate of respiratory viruses in 1165 children hospitalized with bronchiolitis, from January 2018 to December 2021. This observational study confirmed that RSV infection was the most frequent in pre-pandemic years, but dramatically dropped between April 2020-April 2021, as confirmed by reverse transcription polymerase chain reaction (RT-PCR), coincident with the first state of emergency. Other viruses like flu and human metapneumovirus (HMPV) were almost undetectable during this period; non-enveloped viruses such as human bocavirus (HBoV) and human adenovirus (HAdV), although with reduced number of cases, were found more consistently.

After a non-epidemic period, RSV and HPIV (human parainfluenza virus) had a resurgence in the summer of 2021; however no reemergence was detected for HMPV and influenza virus, a fact deserving attention in near future (7).

Considering that most children in their second year of life missed the 2020 bronchiolitis season, due to restrictions, and might have experienced the first episode in following year, children under two years were enrolled in a multicenter prospective study conducted by Camporesi *et al*, in Italy, from 1st July 2021 to 31st January 2022, in the second year of pandemic, outside the lockdowns. The epidemiology, disease severity and microbiology of bronchiolitis episodes were analyzed.

The authors found that the expected season started and peaked earlier than usual, with shorter duration, and that up to 1 in 5 children were older than 14 months, confirming a shift to higher average ages of children affected by RSV. Overall disease severity, however, was similar between the two groups, as well as those with bronchiolitis due to a single virus *versus* multiple viruses (5).

Despite the encouraging results of most published works, global and long-term studies are necessary to achieve more knowledge and provide us better preparation to manage future pandemics and outbreaks.

Fundings

None

Conflict of interests

The authors declare that they have no conflict of interests.

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