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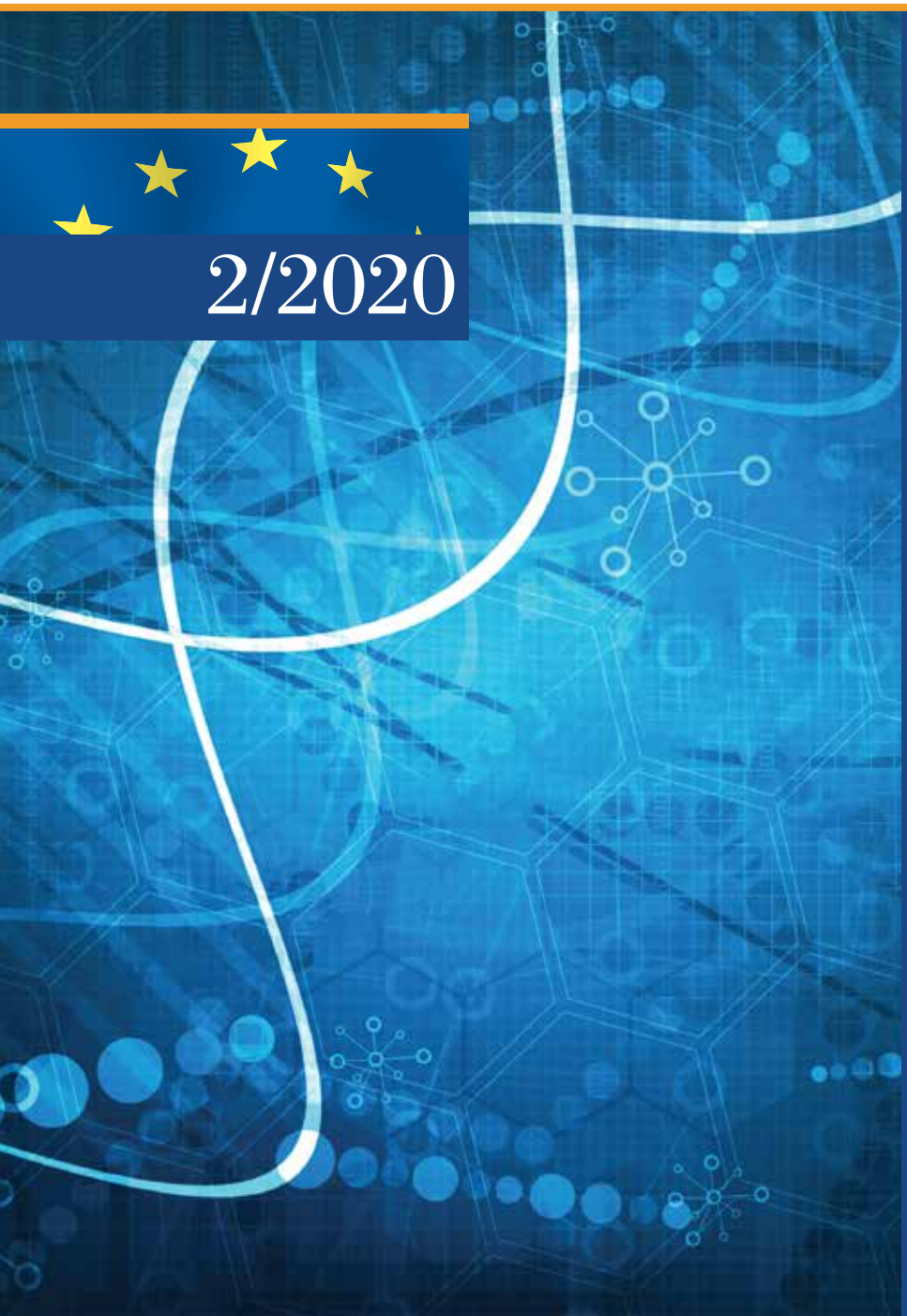

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Recommendations for the use of tryptase in the diagnosis of anaphylaxis and clonal mastcell disorders

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anaphylaxis; tryptase; mastocytosis; hymenoptera sting; post-mortem tryptase

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Summary

Tryptase is a serin-protease produced and released by mast cells after IgE-mediated or non-IgE mediated stimuli. We here review the various aspects related to the molecular characteristics of the enzyme and its biological effects, the genetic basis of its production and the release kinetics. Recommendations for the clinical use of tryptase measurement developed by a task force of Società Italiana di Patologia Clinica e Medicina di Laboratorio and Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri are given on the best procedure for a correct definition of the reference values in relation to the inter-individual variability and to the correct determination of tryptase in blood and other biological liquids, in the diagnosis of anaphylaxis (from drugs, food, insect sting, or idiophatic), death from anaphylaxis (post mortem assessment) and cutaneous or clonal mastcell disorders.

Introduction

Tryptase in its mature form is a neutral serine protease with a molecular weight of 134 kDa. It is present in the secretory granules of mast cells and to a lesser extent in basophils and consists of four beta-tryptase subunits joined by non-covalent bonds and stabilized by proteoglycans. Tryptase is produced in the form of monomer and specifically in the form of alpha, beta, gamma and epsilon subunits. There are two isoforms of alpha-tryptase (alpha I and alpha II) and three isoforms of beta-tryptase (beta I, beta II and beta III) whith high structural identity (around 90%). While the gamma subunit remains bound to the membrane of the secretory granule, alpha and beta monomers are continuously released into the circulation without a specific stimulus and constitute part of the tryptase present in serum (1- 4) (**figure 1**). The mature tryptase is released by the secretory granules as a tetramer composed mainly by beta isoform II. While the monomeric tryptase subunits are practically completely inactive, the mature tetrameric molecule is the active enzyme.

Mast cells, discovered in 1879 by Paul Ehrlich (5), contain many mediators (histamine, serotonin, chimase, carboxypepidase, cathepsin G, proteoglycans, hydrolases and chemotactic factors) in their cytoplasmic granules, but tryptase is the most produced protein and is considered their specific marker (6). The mast cells of the lungs and of the intestinal submucosa contain a higher concentration of tryptase than the mast cells of the skin and of the intestinal mucosa.

Tryptase is also present in basophilic granulocytes, albeit at a much lower concentration (500 times less) than in mast cells, and in very low amounts also in the basophilic precursor cells of the bone marrow (7-9).

The release of tryptase and other mediators from the mast cells is commonly due to IgE mediated immunological stimuli, typical of allergic reactions. In addition to immunological stimuli, physical phenomena such as heat and chemicals (toxins, poisons and drugs, dyes, etc.) can also cause the release of tryptase into the bloodstream.

Genetics

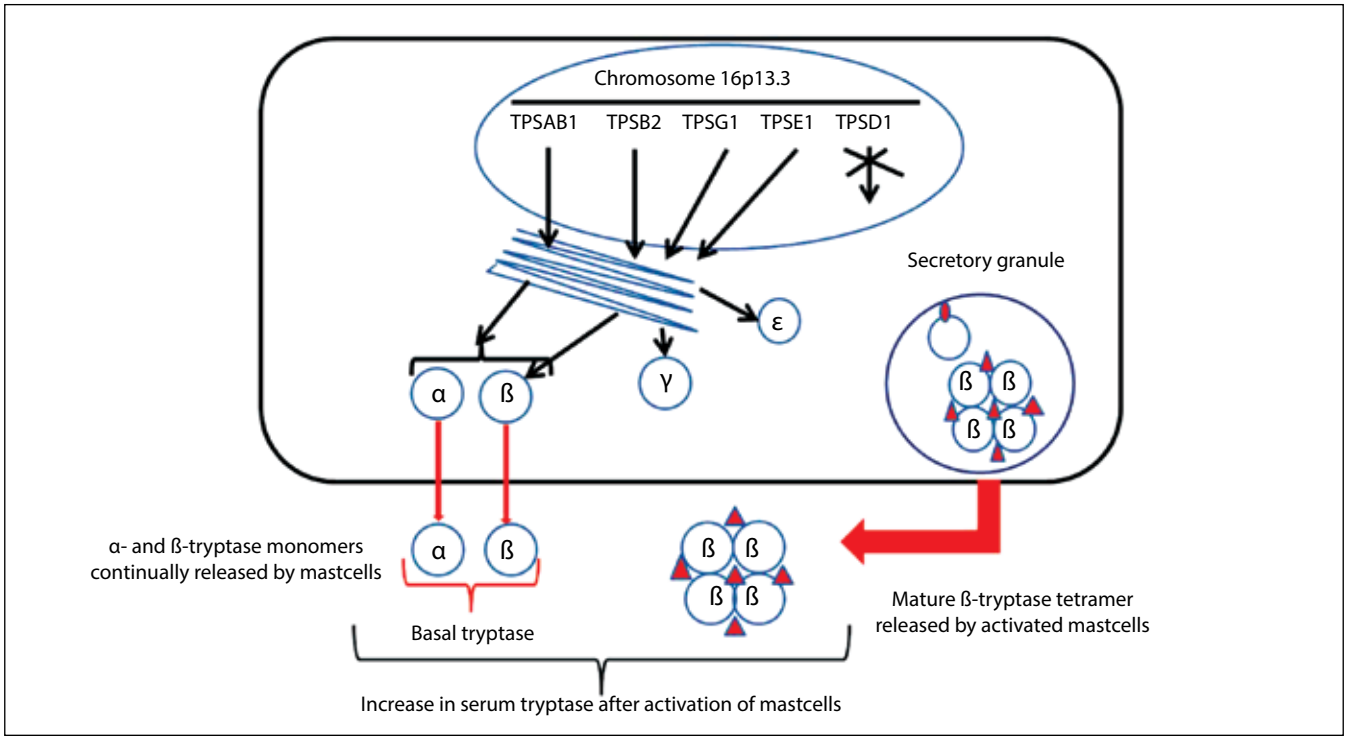
The genes that code for tryptase are located on chromosome 16p13.3. (10-13) and comprise five loci. The tryptase alpha/beta 1 gene (*TPSAB1*) encodes for both alpha and beta I-tryptase, while the *TPSB2* gene encodes for beta-tryptase II and III. The *TPSG1* gene encodes gamma-tryptase and the *TPSE1* gene epsilon-tryptase, a form that is biochemically and immunologically different from alpha and beta tryptase. The *TPSD1* gene, encoding delta-tryptase, is inactive in primates.

The gene that encodes alpha-tryptase is often subject to mutations, which can lead to a transcription deficit or alterations of the catalytic sites, and sometimes a complete deletion (up to 30-57% of the population) (14,15). Also, the polymorphisms of the genes coding for alpha-tryptase and beta-tryptase are high and the number of functional tryptase alleles an individual may carry varies from 2 to 4 (16). Although the absolute deficit of tryptase has never been reported, the number and type of functional alleles carried by an individual may alter the baseline systemic tryptase levels. The frequency of the haplotypes of the two loci of chromosome 16 are 50% for ßß/ßα, 25-29% for ßß/ßß and 21-25% for ßα/ßα (14,17). Although basal tryptase level is considered to be correlated with the amount of mast cells, genetic variations can partly influence the basal value of the enzyme (18-20).

The biological effects of tryptase

Tryptase acts as a vasoactive, proinflammatory, chemotactic molecule, as well as in repairing tissue damage (21-25).

Figure 1 *-Tryptase production and intracellular trafficking. Tryptase basal level is due to a continuous release of the alpha and beta monomeric subunits into the bloodstream. The tetrameric mature tryptase, stabilized by proteoglycans (especially heparin) is released only after mast cells activation. Modified from Vitte J (4).*



In particular, through the production of bradikinins, tryptase promotes vascular permeability and has a chemotactic action on neutrophils and eosinophils, cells involved in the late phase inflammatory allergic reaction. It also stimulates the proliferation of fibroblasts and collagen, contributing to tissue repair and to *restitutio ad integrum* (26,27), and stimulates the proliferation of the smooth muscles of the bronchi. More recently, a role of tryptase in the genesis of pain, such as post-operative pain, has been demonstrated by stimulating protease-activated nociceptors (28).

Tryptase measurement

Several monoclonal antibodies have been developed to measure serum tryptase. The first antibody, defined G5 (29), was able to recognize a linear epitope of the beta isoform with a sensitivity of 2.5 µg/L. Later, other monoclonal antibodies were developed, such as G4 and B12, capable of recognizing both the alpha and beta subunits (30).
The only commercial assay to measure tryptase currently available is the fluoroimmunoenzymatic test (FEIA) (ImmunoCAP, Thermofisher, Uppsala, Sweden), which measures both the immature monomeric forms of the alpha and beta-tryptase and the mature tetrameric form.
Both serum and plasma can be used for the measurement of tryptase (29). The molecule at room temperature is stable for two days (48 hours) and for five days if serum or plasma is stored at 8 °C.

Reference value of serum tryptase

In the last few years the upper threshold of the reference value indicated by the manufacturer for the FEIA test has been lowered several times starting from an initial value of 15 µg/L, then moving to 13.5 µg/L and finally to a value of 11.4 µg/L. This latter cutoff was obtained by the manufacturer evaluating 126 healthy people, in whom the 95th percentile was 11.4 µg/L and the geometric mean 3.8 µg/L.
Schliemann et al (31) in 1092 patients referred to their dermatological service for an allergic / anaphylactic reaction in whom mastocytosis had been excluded, found an average tryptase value of 5.13 ± 3.05 µg/L, a median of 4.46 µg/L and a 95th percentile of 10.8 µg/L. Of these patients, 106 had concentrations >8.75 µg/L and 45 >11.4 µg/L. However, these authors

indicated a slight increase in the value of the tryptase threshold with age progression (95th percentile in subjects between 15-34 years = 9.23 µg/L; between 35-64 years = 10.76 µg/L; >64 years = 12.25 µg/L).
Considering that severe anaphylactic reactions can occur in patients with mastocytosis even with basal tryptase values below 11.4 µg/L (32,33), some authors advise to consider with caution the interval between 8-11 µg/L and to perform also in these cases further investigation to exclude or confirm an underlying mastocytosis.
It is worth mentioning that the reference levels of mature tryptase are <1 µg/L (16), although this has only theoretical importance as we do not have commercial methods able to distinguish tryptase monomers by the mature form.
A very important feature of tryptase is the low intra-individual variability. In fact, the basal value varies very little over time within the same individual and is determined by the genetic background and not by environmental factors (19). This information is useful in the evaluation of anaphylaxis, as even minimal variations in tryptase concentration in a single individual can already be indicative of the presence of an anaphylactic event, even if values fall within the normal range.

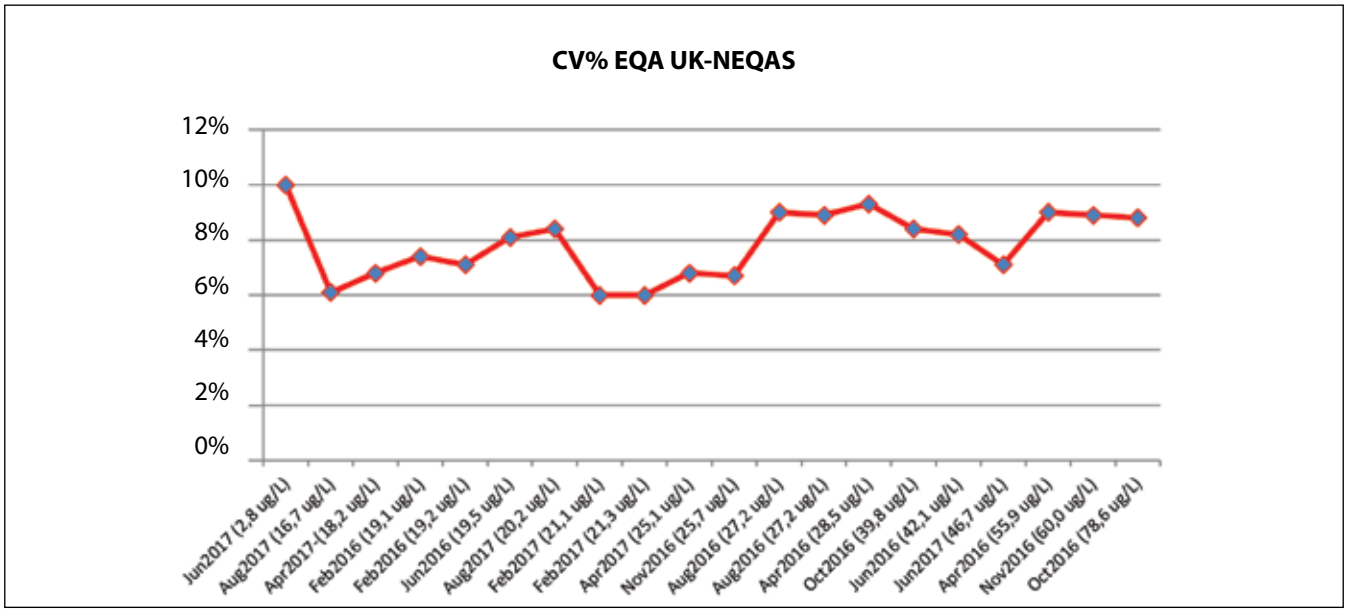
Interference in the measurement of tryptase

Blood samples may be taken in EDTA, heparin or plain tubes without an anticoagulant and preferably analyzed within 5–7 days. Serum tryptase is stable *in vitro*. However, if there is going to be an anticipated delay in analysis, samples must be frozen at –20 °C (34).
Hemolysis, jaundice and lipemia do not appear to interfere with the measurement of serum tryptase. Heterophile antibodies or rheumatoid factors may instead interfere in the tryptase assay (35,36). If an interference by heterophile antibodies is suspected, the latter should be previously removed (31).

Reproducibility of the test

The currently commercially available assay to determine tryptase has good reproducibility, with low intra and inter-assay coefficient of variation (CV) values, as confirmed by Schliemann et al (31) which found an intra-assay variability of 1.7% at 8.26 µg/L and of 1.1% at the value of 44.5 µg/L, and an inter-assay variability of 7.1% at 9.85 µg/L and of 5.5% at the value 33.16 µg/L.
The good reproducibility of the test is also confirmed by results of the UK-NEQAS external quality assessment. The global variability within the 20 control sera with values in the range of 2.8 µg/L - 78.6 µg/L, distributed in the period February 2016 - August 2017 (about 200 participants) showed CVs between 6 and 10%, with an average of 7.8% (**figure 2**).
These data were also confirmed by a study of Davson et al (37) in which 28 samples with tryptase values between 3.3 - 127 µg/L were sent to 25 different laboratories. The average CV of all samples was 8% (range 4.4-12.7%).

Figure 2 *- Variation coefficients (CV) obtained in the UK-NEQAS external quality assessment (EQA) for different tryptase values (period February 2016-August 2017; about 200 participants).*



When is it indicated to test for tryptase?

Tryptase measurement is indicated in the diagnosis of following conditions:

1. idiopathic anaphylaxis or anaphylaxis caused by drugs, food, insect sting;
2. fatal anaphylaxis (*post-mortem* assessment);
3. mastocytosis and mast cell activation syndromes.

Tryptase and anaphylaxis

General considerations

Tryptase is useful for a correct diagnosis of anaphylaxis since similar symptoms may also be present in the vaso-vagal reaction, in septic and cardiogenic shock as well as in the carcinoid and benign flush.

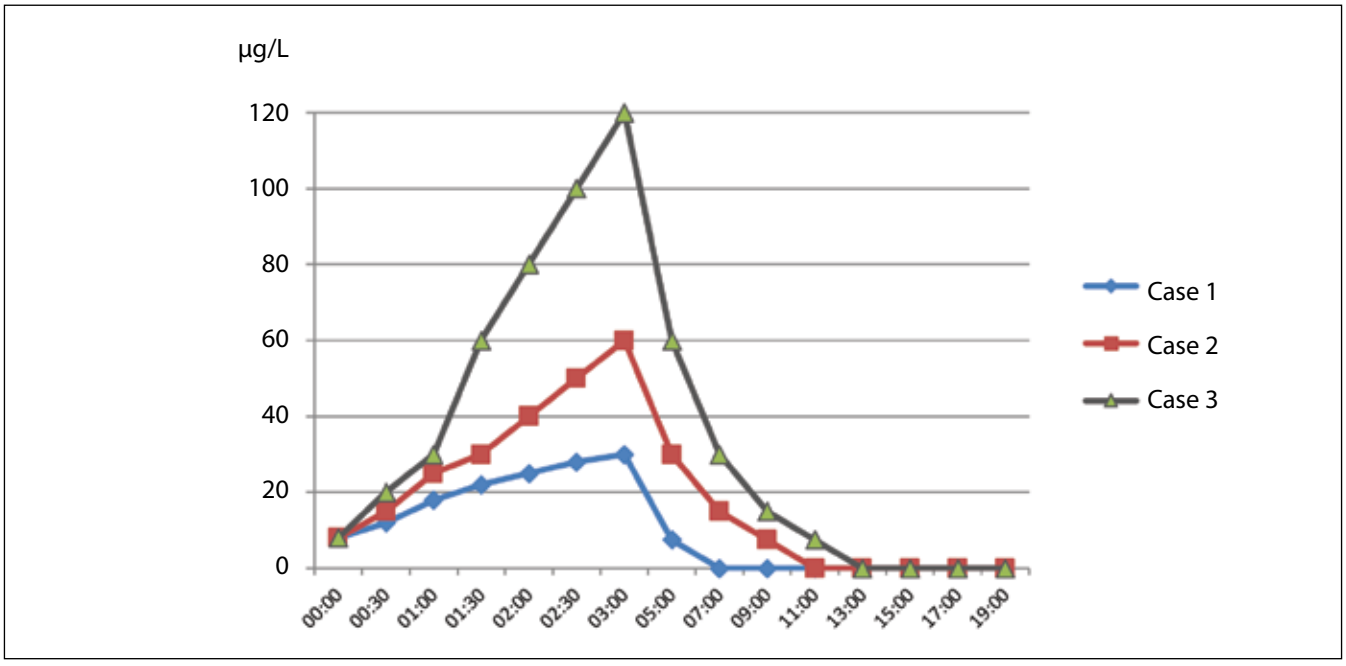
When anaphylaxis occurs, serum tryptase values begin to rise about 5 - 30 minutes after the event, reach the peak after 1-3 hours and return to the basal value within 16-24 hours from the end of the event. The half-life of tryptase is about 1.5-2.5 hours. In **figure 3** the kinetic of tryptase is simulated in three patients with an anaphylactic reaction and with different basal values of tryptase. Even in patients with peak values >100 µg/L, tryptase returns to basal values within 24 hours after the end of the event. However, we must bear in mind that in anaphylactic reactions in which the main involved effector cell is not the mast cell, tryptase may not rise. Moreover, the method to measure tryptase is not an immediate procedure, so results will not be available during the acute episode of anaphylaxis.

In a study in which 30 patients with anaphylaxis were evaluated, Enrique et al (38) showed that using an initial cutoff of 13.5 µg/L proposed by the manufacturer, only 35% (= sensitivity) of the patients had values above this threshold, with a specificity of 92.3%. The sensitivity increased to 94.2% using a cutoff value of 8.2 µg/L, without losing specificity. Similar data were also obtained from other authors (39,40). This indicates that both the value of 13.5 µg/L and the one currently proposed by the manufacturer (11.4 µg/L) are too high to guarantee a sufficient sensitivity of the test in case of anaphylaxis. Various authors have come to the conclusion that the individual increase from the basal tryptase value is more sensitive than the absolute value and this is also supported by the fact that the basal values of an individual remain stable over time.

In consideration of what previously reported, Valent et al (41) suggest that values above 120% of the baseline value + 2 (baseline value x 1.2 + 2) should be considered significant for an anaphylactic event. This International consensus equation has been recently validated (42,43), and it is somehow innovative as it relativizes the concept of a fixed reference value for tryptase.

In order to be able to attribute significance to a value, however, given the speed with which tryptase values fall within the norm after a triggering event, the timing of blood withdrawals is ab-

Figure 3 *- Kinetics of tryptase in three patients with different maximum values.*



solutely important and the training of the Emergency Room personnel is fundamental.
Brown et al (40) have shown that in patients referred to the emergency department for suspected anaphylaxis of whom no previous basal tryptase value was available, a change in value of 2.0 µg/L between two samples spaced one hour apart from the other had a sensitivity and specificity for the diagnosis of anaphylaxis of 73% and 91%, respectively.
Persistently elevated tryptase values after an anaphylactic reaction justify the expansion of the diagnosis for the search for mastocytosis, as indicated by the recent guidelines (44).

Tryptase characterstics in different types of anaphylaxis

a. Drug and anestethic -induced anaphylaxis
Drugs and anesthetics (especially muscle relaxants) are among the main causes of anaphylaxis. In these cases the highest levels of tryptase are often observed, especially when the drugs are administered intravenously (45,46).
Particular attention must be paid to patients who have experienced a drop in blood pressure during general anesthesia, and acute tryptase levels varies as a function of the clinical severity of anaphylaxis (46). However, these events need to be differentiated from non immunological anaphylaxis caused by an isolated histamine release due to a rapid injection of anesthetic drugs (especially morphine derivatives).

b. Food anaphylaxis
In food anaphylaxis, tryptase levels are usually lower than those observed in drug-induced anaphylaxis (47,48). Food anaphylaxis has a slower development and mast cell degranulation is often limited to the intestinal mucosa. In food anaphylaxis, besides release from mast cells, other pathogenetic mechanisms can also be involved, such as a release of basophilic granulocyte mediators or activation of complement factors (C3a and C5a) and kinins. In food anaphylaxis, therefore, the basal value formula x 1.2 + 2 can be particularly useful, since tryptase values are often not very high.

c. Anaphylaxis caused by hymenoptera stings
The literature underlines that there is a preferential association between venom anaphylaxis and elevated basaline serum tryptase level as well as clonal mastcell disorders (49). Rueff et al (50,51) found a close correlation between basal tryptase values and the risk of developing serious systemic reactions after after a hymenoptera sting, as well as in the induction phase of venom immunotherapy. These authors have shown that even values >5 µg/L are related to a greater risk of anaphylaxis, and that the risk increases with the increasing value of basal tryptase, especially in the older age (52).
It is noteworthy that mastocytosis in most cases is only discovered after a person has experienced an adverse reaction following a sting by hymenoptera. Indeed, Bonadonna et al (53) showed that, if the basal tryptase value is >11.4 µg/L and anaphylaxis occurs after a hymenopteran sting, in 88% of the cases we are faced with mastocytosis and that, in the presence of basal tryptase >11.4 µg/L and a negative specific IgE assay for hymenoptera (bee and vespids), the probability of an underlying mastocytosis is 100%.
On the other side, even in the presence of normal tryptase level, patients with severe anaphylaxis (and absence of urticaria or angioedema) due to sting may suffer from clonal mastcell disorders (so called "bone marrow mastocytosis", a subvariant of systemic mastocytosis with a lower burden of clonal mastcells) (33).
Accordingly, a recent consensus by the Italian allergy societies on the management of hymenoptera venom allergy states that, in case of systemic reactions, tryptase determination should be always performed in the diagnostic work up (54). In the presence of persistently high tryptase values, life-long venom immunotherapy is recommended, even if the diagnosis of mastocytosis has not been confirmed (55-57).

d. Idiopathic anaphylaxis
Idiopathic anaphylaxis or spontaneous anaphylaxis is a diagnosis of exclusion and mandates careful consideration of all recognizable and rare causes of anaphylaxis (58).
Idiopathic anaphylaxis represents an opportunity for identification of hidden allergens, cofactors, previously unrecognized novel triggers and also for identification of mastocytosis or clonal mast cell disorders. Other differential diagnoses include "allergy-mimics" such as asthma masquerading as anaphylaxis, undifferentiated somatoform disorder, panic attacks, globus hystericus, vocal cord dysfunction, scombroid poisoning, vasoactive amine intolerance, carcinoid syndrome and phaeochromocytoma (58). Acute serum tryptase measurements are invaluable in patients reporting recurrent episodes and for differential diagnoses.

Fatal anaphylaxis and use of post-mortem tryptase

Post-mortem measurement of tryptase have been reported since 1991 (48), suggesting the possibility to perform a *post-mortem* diagnosis of anaphylaxis, as well as to classify some unexplained deaths as due to anaphylaxis, including some sudden deaths in the pediatric age (59-61). Although this concept may still find its validity, however, it should be borne in mind that *post-mortem* levels of tryptase have been found to be high also in people who died of severe trauma, myocardial infarction, asphyxia, or lung disease. Over time, therefore, the *post-mortem* tryptase cutoff value considered indicative of anaphylaxis as a possible cause of death increased from 44.5 µg/L (61) to 110 mg/L proposed by McLean-Tooke et al (62) in 2014. These authors have evidenced that increased values of tryptase in *post-mortem* sera are quite

frequent even if the cause of death is different from anaphylaxis and only values >110 µg/L have a high diagnostic efficiency for anaphylaxis. However, subsequent studies have again proposed lower thresholds and to date a definitive consensus has not yet been reached (63-65).

Given that data regarding utility of tryptase measurement largely come from case studies or case series (with small sample sizes) and multiple variables increase the uncertainty of measurement when serum samples are obtained from cadavers, to date there is no standardized international reference range for post mortem tryptase (34).

Summarizing all the aspects related to tryptase mentioned above, the intra- and inter-individual characteristics, the kinetics during anaphylaxis, the stability of the basal values, the analytical variability, in the suspicion of anaphylaxis the following is recommended:

Recommendations for the determination of tryptase in anaphylaxis

1. Make the first blood withdrawal preferably 30 minutes – 3 hours after the event.
   - Although the increase in tryptase may already be present after 5-20 minutes after the event, it is advisable to carry out the test after at least 30 minutes to avoid false negative results.
   - In patients with known basal value (not a frequent event), compare the value obtained with the basal value. If the value is 120% higher than the baseline value + 2 µg/L anaphylaxis is confirmed.
   - Consider that values above the cutoff may already be indicative for an anaphylactic event.
2. If possible, take a second sample 1-6 hours after the event to evaluate the kinetics.
3. Make another blood withdrawal at least 24 hours after the event (better after 42-78 hours). This is considered as a baseline value and serves to compare the data to that obtained within 3 hours of the event.
4. Always perform tryptase measurement in subjects who have experienced an anaphylactic reaction following a hymenoptera sting even if the event occurred 24 hours before. High basal values should suggest a mastocytosis.
5. Tryptase can also be determined in *post-mortem* sera when the cause of death is uncertain. In this case it should be remembered that many variables have to be carefully factored into the process of interpretation, including that causes of death other than anaphylaxis can determine an increase in tryptase and only very high values can be indicative of anaphylaxis as the cause of death.

The role of tryptase in clonal mastcell disorders

In maculopapular cutaneous mastocytosis and in mastocytoma the levels of tryptase are usually not increased. Therefore, when a persistent increase in tryptase is observed, the presence of systemic mastocytosis must always be evaluated. In cases of cutaneous mastocytosis of the pediatric age, if a high value of tryptase (>20 µg/L) is present in the absence of systemic involvement, it can be attributed to the release of tryptase from mast cells of the skin. However, it is strongly recommended to monitor the level of tryptase over time. If the level decreases during puberty it is not necessary to perform a bone marrow evaluation.

In diffuse cutaneous mastocytosis, persistently high values, however, must lead to a more in depth diagnostic work up in order to rule out systemic mastocytosis.

Serum tryptase is the most specific laboratory marker for the diagnosis of systemic mastocytosis. As already reported above, the test was included among the minor criteria for diagnosis. Since patients with systemic mastocytosis usually have basal values of tryptase >20 µg/L, it is essential to measure the protein away from events caused by the release of mediators from the mast cells and it is also necessary to check the concentration of serum tryptase at least a second time to confirm a persistent rise of the enzyme.

It should be noted that the absolute value of serum tryptase does not indicate the type of mastocytosis (49). Mastocytosis associated with a hematological disease and aggressive forms of mastocytosis may have values similar to the indolent form (66). In mastocytic leukemia, however, the values are usually extremely high and can reach levels as high as >1000 µg/L.

In the mastocytosis with associated hematologic disease subtype, the criterion of tryptase is not applied since the high value of the enzyme can also derive from the precursor cells of the bone marrow.

Other pathologies that can determine an increase in tryptase

High tryptase values were detected in patients with acute myeloid leukemia (67), myelodysplastic syndrome (68), hypereosinophilic syndrome (associated with the FIP1L1 PDGFRA mutation) (69), terminal renal failure (70), in abdominal aortic aneurysm (71), in some forms of infestation with helminths (72) and, in rare cases, a genetic increase in the family has been described (73,74). In particular, familial tryptasemia is a recetly described disease in which members of the same family present elevated baseline tryptase levels due to hereditary alpha-tryptaseaemia (autosomal dominant) due to increased germline copies of alpha-tryptase gene (TPSAB1) (73,75). These patients have an elevated baseline tryptase with or without non-specific multisystem symptoms. This condition has not yet been well-characterized (75).

Recommendations for the determination of tryptase in suspected mastocytosis

1. *Determination of tryptase in suspected infantile mastocytosis.*
   - Normal tryptase values do not exclude mastocytosis.
   - When tryptase values are <20 µg/L, with absence of mediator release symptoms and of hepatosplenomegaly or enlarged lymph nodes or changes in blood count, it is not necessary to proceed with bone marrow biopsy.
   - Values >20 µ/L and/or mediator release symptoms and/or hepatosplenomegaly, are indications to proceed with bone marrow biopsy.

2. *Determination of tryptase in suspected clonal mastcell disorders in adults.*
   - In the suspicion of an adult mastocytosis it is recommended to always measure tryptase, since the value is often increased. However, it should be noted that the value is not indicative of a specific clinical form of mastocytosis, with the exception of mastocytic leukemia which has very high levels.
   - In anaphylactic reactions due to insect sting it is recommended to always measure tryptase, because of a possible underlying indolent systemic mastocytosis. In all cases, a high tryptase value should be confirmed with a second test after at least 3-4 days.

In acute myeloid leukemia, high levels of tryptase, produced by precursor cells, were observed in 40% of patients. In myelodysplastic syndromes, tryptase is synthesized specifically by atypical mast cells. In hypereosinophilic syndromes with FIP1L1 PDGFRA mutation, mast cell hyperplasia is the cause of increased tryptase.

Tryptase measurement on fluids other than blood

Besides serum, tryptase can be measured in other biological fluids, such as the broncho-alveolar lavage fluid, the intestinal fluid, the nasal and the lacrimal secretion. In basal conditions, its determination in the nasal fluid can be particularly useful for monitoring inflammation *in situ* and high concentrations are characteristic of allergic rhinitis.
During the provocation tests for allergic diseases, high levels of tryptase can be found in all the aforementioned materials. After nasal challenge the mast cells release histamine and preform tryptases and this determines an increase in their concentration in these secretions (76), together with that of prostaglandin (PGD2) and cysteinyl leukotrienes (Cys-LT). The level of mediators released after nasal challenge is extremely variable from individual to individual. In particular, in atopic responders, tryptase levels can increase up to seven times the baseline value, a much higher rate than for histamine (77). Furthermore, it has been shown that the increase in tryptase in the nasal secretion appears to be particularly significant in patients with perennial allergy to Dermatophagoides pteronyssinus (78).
Tryptase measurement, both in basal conditions and after allergenic challenges, can be performed using the nasal microaspiration technique that allows a quantitative measurement of mediators in secretions even with small volumes of appropriately diluted materials (79). Nasal sticks are especially useful in pediatric age (80). The collected material can be processed, with or without any dilution (after washing the solid phase with NaCl 0.9% and Tween 0.05% 3 times for 5 minutes) with the fluoroimmunoenzymatic method (FEIA) similarly to what is done for serum dosage.
Less used, but equally possible, are measurements in the lacrimal secretion, saliva and bronchial and intestinal lavage fluids. The presence of tryptase in tears is due to the release by conjunctival mast cells in patients with allergic inflammation. In particular, the level of tryptase is high in the acute phase of the reaction, but not in the late one. Finally, in subjects in whom the presence of a food allergy is suspected, it can be useful to measure tryptase in the saliva before and after a challenge test with the offending food (81).

Conclusions

Measurement of blood tryptase is a simple test, with a good reproducibility, within the reach of all laboratories, and it is very useful for the diagnosis of all forms of mastocyte activation. Its greatest usefulness is in the diagnosis of anaphylaxis, when the clinic it is uncertain or may be compatible with other causes, as well as in the diagnosis of mastocytosis. Therefore, this tool should be available in all laboratories. Thanks to tryptase dosage, often performed after an anaphylactic episode following an hymenoptera sting, many forms of mastocytosis, especially in the indolent form, can now be identified. This highlights the need for integrated work among allergists, hematologists and clinical pathologists in the diagnosis of these diseases.

Conflict of interests

The authors declare that they have no conflict of interests.

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Development and validation of the parent-reported drug hypersensitivity quality of life questionnaire

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Key words
drug hypersensitivity; parent-reported; quality of life; allergy; well-being

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Summary

Background. *Drug hypersensitivity in children impacts the quality of life of the patients and their caregivers. Measurements of the quality of life in children are different from adults, because children cannot answer the questions. This research aimed to develop and validate the Parent-reported Drug Hypersensitivity Quality of Life Questionnaire (P-DrHy-Q).* **Methods.** *The 21-item scale was initially generated by researchers. Then, 3 experts were asked for their opinion about the scale. After adjusting the contents and language, the scale was answered by 97 caregivers. A factor analysis was carried out to select the items for the final scale, and Cronbach's alpha assessed the internal consistency. Finally, we examined the test-retest reliability in another group of 10 caregivers.* **Results**. *The 21-item scale was grouped into 6 factors. However, some factors were inappropriate. Therefore, the number of factors was reduced using a statistical analysis. The final 12-item scale included two factors: mental health and social activity. The scale had good internal consistency (Cronbach's* $\alpha = 0.897$*) and the test-retest associations were good (*$R = 0.9439$; $p < 0.001$*).* **Conclusions**. *The P-DrHy-Q is the first scale for assessment to consider the interaction of biopsychosocial factors on drug allergy that includes the carer-child dyad. It shows good internal consistency and reliability. Its application might be relevant for future research, and provide clinicians and researchers with a solid tool to define which type of psychosocial support is required to provide more comprehensive care in drug hypersensitivity.*

Introduction

Drug hypersensitivity is a term suggested by the Review Committee of the World Allergy Organization that refers to "objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons" (1) independently of the pathogenetic mechanism. Hypersensitivity reactions to drugs affect 10% to 20% of hospitalized patients and more than 7% of the general population (2,3).

Drug hypersensitivity affects not only the physical health but also the mental health and quality of life of a patient and all family members. The Drug Hypersensitivity Quality of Life Questionnaire was initially created by Baiardini I et al. (4) and the results showed good validity, internal consistency, and reliability. However, measuring the quality of life in children is different from adults. Children cannot answer the questions on their own and the measurements must rely on the answers from the caregiver.

Previously, a quality of life questionnaire was established to measure the caregivers who have children with allergic disease such as asthma (5,6) and food allergy (7,8). To date, no questionnaire is available to measure the quality of life in caregivers who have children with drug hypersensitivity. Therefore, we aimed to develop a questionnaire for the specific burden of drug hypersensitivity from the caregiver's perspective. Our goal was to develop a tool that would capture the health-related quality of life (9) using a multi-dimensional concept to examine the impact of the health status on the quality of life of caregivers who have children with a history of drug hypersensitivity. We have named this new tool the Parent-reported Drug Hypersensitivity Quality of Life Questionnaire (P-DrHy-Q).

Methods

Ethics

Ethical approval for development and validation of this study was provided by the Office of Human Research Ethics Commit-

tee at Prince of Songkla University. All participants gave written informed consent to participate.

Item generation and validity

The researcher, a pediatric allergist, and immunologists generated a 21-item questionnaire using the Food Allergy Quality of Life Questionnaire as a reference. Each item consisted of a visual analog scale with a 0 - 10 point scale. We then asked for the opinions of three experts: a pediatric allergist and immunologist; an internal medicine allergist and immunologist; and a developmental-behavioral pediatrician. The questionnaire was adjusted by recommendation and then 97 caregivers of children under the age of 15 years with a history of drug hypersensitivity answered the P-DrHy-Q at our pediatric outpatient department. The questionnaires were collected from Oct 2016 to March 2017. After the data collection was complete, a factor analysis was done to group the questions.

Reliability

The scale homogeneity (internal consistency) was computed based on Cronbach's correlation coefficient on the extracted factors. Cronbach's alpha analysis was used for the full scales and subscales. The test-retest reliability of the P-DrHy-Q was ascertained to determine whether the questionnaire would provide the same results when used repeatedly in a stable condition. Therefore, 10 caregivers of children under the age of 15 years with a history of drug hypersensitivity answered the P-DrHy-Q two times at the pediatric wards at 3 days apart in the absence of any significant clinical or personal change. The results were analyzed by Pearson's correlation coefficient.

Statistical analysis

The data were recorded using Epidata and analyzed using R statistical software. The internal consistency of the scale was evaluated using Cronbach's alpha coefficient. It is accepted crossways that alpha > 0.7 is acceptable, > 0.8 is good, and > 0.9 is excellent. It was acceptable at a loading level greater than 0.5. Pearson's correlation coefficient test was performed to assess the discriminative reliability of the test-retest associations.

Results

Item generation and validity

The researchers generated a 21-item questionnaire. After obtaining the opinions from the experts, the questionnaire was adjusted. The adjusted questionnaire was applied in 97 caregivers of children under 15 years of age with a history of drug hypersensitivity at the pediatric outpatient department. The demographic characteristics of the participants are summarized in **table I**. After the data collection, 21 items were included in the factor analysis. Varimax factor rotation was undertaken and only factors that were more than 0.50 were considered for the analysis. Six components were extracted using the principal component analysis. However, due to item distribution, the analysis was forced into two factors and each of the items had a loading level greater than 0.50. The final version of the

Table I *- Demographics for participants in validate phase (n = 97).*

Sex female, number (%)	84 (86.6)
Current age (yr), median (range)	39.1 (22.3 - 72.4)
Main caregiver, number (%)	
father	13 (13.4)
mother	76 (78.4)
other	8 (8.2)
Informative caregiver, number (%)	
father	14 (14.4)
mother	79 (81.4)
other	4 (4.1)
Marital status, number (%)	
living with partner ≥ 6 mo/yr	86 (88.7)
living with partner < 6 mo/yr	4 (4.1)
devoid	3 (3.1)
widow	2 (2.1)
Occupation, number (%)	
government officer	27 (27.8)
state enterprise	4 (4.1)
owner	8 (8.2)
officer	15 (15.5)
homemaker	16 (16.5)
freelance	27 (27.8)
Education, number (%)	
primary school	7 (7.2)
high school	16 (16.5)
certificate	10 (10.3)
bachelor	53 (54.6)
master/PhD	11 (11.3
Number of children within family,	
number (%)	74 (76.3)
1-2 persons	23 (23.7)
3-4 persons	0
> 5 persons	
Family income Baht/month, number (%)	
< 15 000	31 (32.0)
15 000 - 50 000	49 (50.5)
50 000 - 100 000	14 (14.4)
> 100 000	3 (3.1)
Experienced in care children with history of drug hypersensitivity before, number	
(%)	62 (63.9)
no	35 (36.1)
yes	

Figure 1 *- Parent-reported drug hypersensitivity quality of life questionnaire (P-DrHy-Q).*

Within the past week,	For official
1. You had sleep disorder problem due to your child's drug allergy no effect ——— very affected	□□.□
2. Your child's drug allergy affected your mood no effect ——— very affected	□□.□
3. You were worried that your child will be allergic to drug again no worry ——— very worried	□□.□
4. The worry that your child would be allergic to drug again affected you no effect ——— very affected	□□.□
5. The fear of your child would allergic to drug again affected to you no effect ——— very affected	□□.□
6. You worried that your child would have a learning problem due to drug allergy. no worry ——— very worried	□□.□
7. Your child's drug allergy made you frustrated no effect ——— very affected	□□.□

Within the past week,	For official
8. You needed to take care of your child more than usual when you go out. not necessary ——— very necessary	□□.□
9. Your child's drug allergy made you lack the time for leisure activities (exercise, movie, eating out). no effect ——— very affected	□□.□
10. Your child's drug allergy caused your child felt discriminated from another child. no effect ——— very affected	□□.□
11. Your child's drug allergy affected your family budget no effect ——— very affected	□□.□
12. Your child's drug allergy affected your social interactions no effect ——— very affected	□□.□

P-DrHy-Q (**figure 1**) consisted of 12 items distributed into two factors: mental health and social activity (**table II**).

Reliability

Cronbach's alpha coefficient for the P-DrHy-Q was 0.897 which indicated good internal consistency between the individual questions in the instrument. The alphas for all subscales are shown in **table III**. To determine the test-retest reliability of the P-DrHy-Q, 10 caregivers of children under 15 years of age with a history of drug hypersensitivity answered the questionnaire two times at the pediatric wards at 3 days apart. During this period no change in the child's drug hypersensitivity was expected. Pearson's correlation coefficient was 0.94 ($p < 0.01$) which indicated excellent reliability.

Table II *- Factor analysis and loadings.*

Item (abbreviated wording)	mental health loading	social activity loading
developed sleep disorder	0.50	
impacted your mood	0.50	
worried that your child will be allergic to drug again	0.86	
worried that your child being allergic to drug again affects you	0.82	
fear that child being allergic to drug again affects you	0.71	
worry that your child will have a learning problem due to allergy		0.51
child's condition makes you frustrated		0.63
when you go out you need to take care more than usual		0.52
lack of time for leisure (exercise, movie, eating out)		0.74
felt discriminated from another child		0.71
your family budget is affected		0.69
your social interactions are affected		0.61

Table III *- Cronbach's alphas for the P-DrHy-Q and subscales.*

P-DrHy-Q	Cronbach's alphas
Total scale	0.8974
Subscales	
mental health	0.8735
social activity	0.8424

Discussion

The results of the study showed that the P-DrHy-Q is a self-applied psychosocial impact scale in drug allergy. Furthermore, it is a brief and low-cost way to assemble data that may guide the clinician to decide which factors should be included in a multidisciplinary approach to their patients.

The strength of this study is that this is the first scale in drug allergy that focuses on the carer-child dyad. This scale showed good internal consistency and reliability. The factor analysis demonstrated that the scale may be used to measure two types of parental burden: mental health and social activity. Both of these domains had very good internal reliability in both versions of the scale. Therefore, it may be possible to adapt the scale to incorporate two sub-scale scores as well as an overall score to provide more information on the type of parental burden that is most salient. A limitation of the study was that it was done in a single center that possibly did not include all of the patients with different types of drug allergy.

Conclusions

This is the first parent-reported health-related quality of life instrument for drug allergy. This study demonstrated that the P-DrHy-Q is reliable and valid for a Thai population. Factor analysis revealed two distinct domains: mental health and social activity. Gaining information on which type of parental burden is more salient and may be useful in determining appropriate support for the caregivers. A further study to evaluate other psychometric properties is essential.

Conflict of interests

The authors declare that they have no conflict of interests.

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Study on the effect of phototherapy for inhibition of symptoms associated with allergic rhinitis

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Key words

phototherapy; allergic rhinitis; grass pollen; intranasal; allergy

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Abbreviations

Total nasal symptom scores, TNSS, allergic rhinitis, AR.

Summary

Previous published work has indicated that treatment of the inside of the nose with certain wavelengths of light can reduce the symptoms of allergic rhinitis. The objective of the study was to compare the efficacy of the phototherapy device on the relief of a range of symptoms provoked by indoor and outdoor allergens. A phototherapy emits visible light (mUV/VIS) and infrared light, and was compared to a placebo device which did not emit light on two groups of allergic rhinitis sufferers. Rhinophototherapy improved nasal symptoms of allergic rhinitis arising from exposure to indoor and outdoor allergens. The difference in the intensity of symptoms scored at the baseline, and at the final visit for the group using the photoperiod device was significantly lower. The device could potentially help improve the quality of life for allergy sufferers. Phototherapy may be suitable for sufferers either as a replacement therapy or used alongside traditional medication.

Introduction

The nose is the first line of defence against inhaled potentially harmful airborne particles. By acting as a filter, it prevents allergens from reaching the bronchial tree. Allergic rhinitis (AR) results from the inflammation of the nasal lining caused by an allergen, such as pollens, moulds, dust or certain animal danders, which cause symptoms such as nasal irritation, sneezing, rhinorrhoea and nasal blockage (1). These common reactions affect approximately 25% of the population worldwide and can lead to a reduction in the quality of life, with economic impacts (2,3).
AR is often treated using pharmacological products such as antihistamines, corticosteroids or cromolyns either on their own or in a combination depending on the symptoms experienced. However, there are sufferers who do not wish to take medication or for whom medication is contraindicated (4). There are also allergic rhinitis sufferers who wish to reduce the amount of medication that they take, or who find that medication is not sufficient to control their symptoms. One possible method in reducing the dosages of pharmacological products may be to combine their usage with other methods.
Previous published work has indicated that treatment of the inside of the nose with certain wavelengths of light can reduce the symptoms of allergic rhinitis (5). Early studies looked at the effects on perennial / persistent rhinitis and more recent studies (6,7) have looked at the effect on seasonal / intermittent allergic rhinitis. Phototherapy has an immunosuppressive effect and is widely used for the treatment of immune mediated skin diseases. Phototherapy devices are able to inhibit immediate type hypersensitivity reaction in the skin. Intranasal phototherapy is an approach more suitable for treatment of allergic rhinitis. In two open studies, 308 nm excimer laser and topical PUVA therapy efficiently inhibited clinical symptoms of allergic rhinitis (5). In a randomized, double-blind study combined low dose UVB,

low dose UVA and visible light proved to be effective in reducing symptom scores for sneezing, rhinorrhea, nasal itching and the total nasal score in ragweed allergic patients. Light wavelength used in phototherapeutic treatment ranged from red light to ultraviolet. Clinical use of intranasal phototherapy appears to be safe and well tolerated. Most studies demonstrated symptomatic improvement in quality of life scores. Treatment with low-energy narrow-band red light phototherapy was demonstrated to improve symptoms in 72% of the allergic rhinitis patients and the objective improvement was endoscopically demonstrated in 70% of in comparison with 24% and 3%, respectively, which was observed in the placebo group (8). These were significantly different. Intranasal phototherapy may represent an alternative treatment of allergic rhinitis and other inflammatory and immune mediated mucosal diseases.

The study reported here investigated the effect of a phototherapy on seasonal / intermittent and perennial / persistent allergic rhinitis symptoms with sufferers who may be affected by one or more allergen sources.

Methods

Phototherapy test device

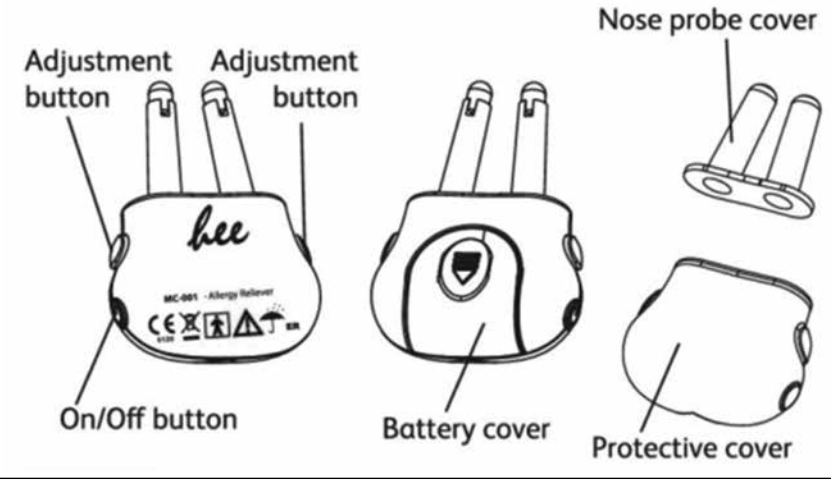
The phototherapy device used in the trial was a Class IIA medical device (Kodec Holdings, Unit D, 20/F., Tai Ping Industrial Centre, Block 1, No 57 Ting Kok Road, Tai Po, New Territories, Hong Kong). The phototherapy device (model Nos mc0018004) has two specific wavelengths which are recommended for reducing the symptoms of Allergic Rhinitis. The device emits visible light (mUV/VIS) and infrared light (660nm8940nm).

The nose probe covers are removed and the on/off button depressed for 1 second, to activate the two wavelengths (**figure 1**). The two nasal probes are inserted into the nasal cavity by pressing the 2 adjustment buttons. The treatment lasts for 3 minutes and the device automatically turns off once the treatment is completed. The device was used by participants for 3 minutes, twice a day, 5 to 6 hours apart. A placebo device which did not emit light was used on the control group. Participants used the active and placebo device in the morning and evening, although participants were able to fit the use into their normal daily schedules. The study was designed so that participants used the device for 3 weeks with readings taken after 2 weeks (mid study visit-MSV) of use and again after three weeks of use (final study visit -FSV).

Study participant characterisation

Data and other sample size calculations from previous studies were used to determine the sample size required for this study (9,10). The study comprised of 52 participants with sensitivity to grass and 50 participants with either sensitivity to cat and/or house dust mite. Participants were provided with a participant information sheet on the nature and scope of the study and were required to submit a signed informed consent form. Inclusions and exclusions were applied. Participants had to be aged 18 years of age or older and sensitive to grass pollen and/or cat dander and/or house dust mite allergen within the previous 2 years. Participants with a history of asthma, nasal deformities / polyposis and sensitive skin were excluded. They were also excluded if they had reported medical conditions or had cold, flu or rhinitis during the initial visit.

Figure 1 *- Phototherapy device (Wavelengths 660 nm8 940nm-Model number mc-0018004).*



Method used for skin prick testing

Potential participants were skin prick tested for their sensitivity to grass pollen, cat dander and house dust mite allergen using standard solutions (ALK 7 Abello Soluprick SQ allergen extract 10 HEP) together with a positive control (histamine hydrochloride, ALK Abello Soluprick 10 mg/ml) and a negative control (saline solution, ALK Abello Soluprick). The criteria for a positive test was the larger of either a wheal with 3 mm mean diameter or a wheal with a diameter of 3 mm greater than the negative control as defined by the World Allergy Organisation (11).

Allergy history

Participants reported their allergic rhinitis symptom history using scoring scales to ensure they were suitable to participate in the trial (**table I**) (12). The participant group had 38 people reporting sensitivity to the outdoor allergen (grass pollen) and one or both of the indoor allergens (cat dander and/or house dust mite allergen), 14 people reporting sensitivity to the outdoor allergen (grass pollen) only, and 12 people reporting sensitivity to the indoor allergens (cat dander and/or house dust mite allergen) only. This showed that there were 52 people with allergy to grass pollen, and 50 people with allergy to cat dander and/or house dust mites (**table II**). Details of the gender and age breakdown of participants is also shown on **table II**. At the start of the trial no participant was showing any symptoms associated with allergic rhinitis.

Methods of assessing participant nasal symptoms and participant baseline readings for the trial

As the trial was conducted during the period of the year when grass pollen was not present, participants were not using allergy medication. Study participants allergic to cat/house dust mite were asymptomatic at the start of the trial and were not using medication. No trial participants were undergoing immunotherapy. Previously reported methods were used to study nasal symptoms in the trial reported here (13,14). The sum of the Total Nasal Symptom Score (TNSS) is an established method for determining symptom levels of allergic rhinitis. This involves

Table II *- Allergen sensitivity, gender and age of participants in the photoperiod study.*

Allergen	**Number in study**
outdoor (grass) only	14
indoor (cat/house dust mite) only	12
indoor and outdoor (grass and cat/house dust mite)	38
total in study	64 (26 males / 38 females)
Allergen	**Number in study**
outdoor (grass)	52
indoor (cat/house dust mite)	50
Age characteristics of participants	**Number**
18 - 25 years	24
26 - 35 years	14
36 - 45 years	15
46 - 55 years	6
56 - 65 years	4
65+ years (average age 33.7 years)	1

Table I *- Criteria for assessing allergy history of participants.*

Symptom	**Score**	**Criteria**
scoring of runny nose	(0 - 3)	nasal blowing (0 - 10+ daily episodes)
scoring of itchy nose	(0 - 3)	rubbing nose (0 - 10+ daily episodes)
scoring of blocked nose	(0 - 3)	nasal stuffiness and mouth breading
scoring of sneezing	(0 - 3)	sneezing (0 - 10+ daily episodes)
itchy eyes	(0 - 3)	rubbing eyes (0 - 10+ daily episodes)
watery eyes	(0 - 3)	watering eyes (0 - 10+ daily episodes)
itchy throat	(0 - 3)	itchy throat (no itching to very itchy)
itchy mouth	(0 - 3)	itchy mouth (no itching to very itchy)
itchy ears	(0 - 3)	itchy ears (no itching to very itchy)

evaluating the intensity of nasal symptoms (runny nose, itchy nose, blocked nose, and sneezing) on a scale from 0 to 3 (0 = no symptom, 1 = mild, 2 = moderate, 3 = severe). The TNSS was obtained from the sum of all 4 individual symptom scores, with a total possible score ranging from 0 (no symptoms) to 12 (maximum symptom intensity). Other symptoms recorded were ocular (itchy eyes, runny eyes) and other allergic symptoms (itchy mouth, itchy throat, itchy ears) using the same scale of intensity as used in the TNSS score.

Method of allergen exposure

A controlled environment test chamber was used in the studies during exposure to allergens. The chamber was set to a typical summer's day with an ambient temperature of 20 °C with a humidity of 50%. A self-contained allergen challenge chamber which was used to replicate different conditions was located within the environmental test chamber. Previous studies have established allergen challenge chambers as being suitable for studies using allergens (15-17).
Before entering the chamber, each participant was required to put on protective clothing (laboratory coat, hair net, shoe protectors, gloves) to prevent allergen from escaping from the chamber. A tube containing a pre-weighed amount of Timothy grass (*Phleum pratense*) pollen grains (supplied by Allergon, Denmark) was fitted to the dispersal mechanism. Timothy grass pollen counts can reach between 150 and 400 pollen grains per cubic metre in the UK during summer. Previous studies with grass pollen established that 150 and 400 pollen grains per cubic metre of air are equivalent to high pollen count days in summer. The number of pollen grains required to replicate these field conditions were approximately 6000 grains. Cat dander and house dust mite allergen used levels to replicate equivalent conditions in a typical household and provoke symptoms (18). This equated to approximately 500 particles of both house dust mite (25 µg/g Der p1) and cat dander (14 µg/g Fel d1) within the chamber. After 15 minutes the participants left the allergen challenge chamber.

Randomisation

A random number generator was used to determine the allocation of groups for treatment or placebo group. Participants over the age of 50 were stratified between the treatment group and placebo group as 60% of rhinitis patients over the age of 50 have symptoms from a non-allergic cause (19). All participants were blinded to the group they were allocated until the end of the study. The study population was made up of 26 males and 38 females. The details of the sensitivity of the participants to different allergens in the treatment and placebo groups are shown in **table III**.

Table III *- Allergen sensitivity breakdown for the treatment group and placebo group.*

Allergen	number in treatment group	number in placebo group	Total
outdoor (grass) only	6	8	14
indoor (cat/house dust mite) only	5	7	12
indoor and outdoor (grass and cat/house dust mite)	19	19	38

Recording participant symptoms during the study

Mid study visit (MSV)
At the mid study visit, participants had baseline readings taken and then spent 15 minutes in the chamber as per the protocol for the baseline visit. They then had their symptoms monitored for an hour afterwards using the TNSS scale (14).

Final study visit (FSV)
At the final visit, participants had baseline readings taken and then spent 15 minutes in the chamber as per the protocol for the baseline visit. They were then had their symptoms monitored for an hour afterwards using the TNSS scale (14).

Statistical analysis
Mann Whitney-U test was used to determine significance ($p \leq 0.05$). All statistical tests were carried out two-tailed at 5% significance levels.

Results

Effect of phototherapy on eye and nose allergic reactions

No serious adverse effects were reported either during or after the study from the participants using the protocol applied. Two participants reported that they had severe rhinorrhoea while using their devices, however both of these participants were in the placebo group. One participant reported a faulty device but this was immediately replaced. No problems with using the devices were reported. No problems with compliance with the protocol were reported.

Participant baseline analysis

A total of 64 data sets were collected. There was a good relationship between the symptoms reported by the participants in their allergy histories and symptoms provoked in the Allergen Challenge Chamber during the baseline visit. There was no dif-

ference in allergic reactions between groups irrespective of type of allergen used in the allergen challenge (**table IVa**).

Total nasal symptom scores (TNSS) at final visit

The TNSS (runny nose, itchy nose, blocked nose, sneezing) was obtained from the sum of all 4 individual symptom scores, with a total possible score ranging from 0 (no symptoms) to 12 (maximum symptom intensity). The total TNSS for the placebo group at baseline was 237 (**table IVb**), with an overall mean of 7 (SD = 2). The total TNSS for the treatment group at the first visit at the beginning of the trial was 220, with an overall mean of 7 (SD = 2). There was no significant difference in the TNSS for the treatment group and the placebo group at the first visit at the beginning of the trial (p = 0.25014). There was no significant difference in the TNSS for the treatment group and the placebo group at the first visit at the beginning of the trial for the different categories of allergen (**table IVb**). The total TNSS for the placebo group at the final visit was 209, with an overall mean of 7 (SD = 2). The total TNSS for the treatment group at the final visit was 142 (**table IVb**), with an overall mean of 4 (SD = 2).
The TNSS showed that there was little change in the intensity of symptoms scored at the baseline and at the final study visit for participants in the placebo group (p = 0.09492); with only a slight change in numbers at each intensity level. The difference in the intensity of all symptoms scored at the baseline and at the final visit for the group using the photoperiod device was significantly lower (p = 0.00024***) (**table IVb**) with a reduction in the intensity of symptoms (**table V**). The effect of the photoperiod device was observed mainly in the total nasal symptom scores (TNSS). Sensitivity to grass represented the major allergenic response group in the trial.

Table V *- TNSS symptom intensities for the placebo and treatment group at baseline and final visit.*

	Placebo group numbers		**Treatment group numbers**	
TNSS symptom intensity	number at baseline	number at final visit	number at baseline	number at final visit
very mild (0 - 2 points)	1	1	0	7
mild symptoms (3 - 5 points)	5	8	7	14
moderate symptoms (6 - 9 points)	21	20	19	11
severe symptoms (10 -12 points)	5	3	6	0
total participants	32	32	32	32

Nasal symptom scores for each allergen sensitivity group

The outcomes for the different sensitivity groups followed a similar pattern to the overall study
(**table VIa** and **VIb**). There was a consistent decrease in the TNSS scores from the baseline visit to the final visit across the three allergen groups (**table VIa**). This was not observed in the

Table IV *- Comparison of treatment and placebo group for a) participant number and mean nasal symptom score with sensitivity type b) TNSS at baseline and final visit for all sensitivities.*

a)

allergen type	**number in placebo group**	**number in treatment group**	**mean score placebo group**	**mean score treatment group**	**p value**
grass only	8	6	7	7	0.60306
grass and cat/house dust mite	18	21	7	7	0.68916
cat/house dust mite only	6	5	7	8	0.20054

b)

severity scores	**baseline placebo group**	**final visit placebo group**	**baseline treatment group**	**final visit treatment group**	**p value**
TNSS	237	209	220	142	
overall mean score	7	7	7	4	0.00024***

*** highly statistically significant

Table VI *- Comparison of mean score and Total TNSS for a) placebo and treatment groups at baseline and final visit with allergen type, b) p values for the TNSS between groups.*

a)

Placebo group				
allergen type (baseline)	**mean score**	**mean score (final visit)**	**total TNSS score (baseline)**	**total TNSS score (final visit)**
grass only	7	6	57	46
grass and cat house dust mite	7	7	123	120
cat/house dust mite	8	7	58	43
Treatment group				
allergen type (baseline)	**mean score**	**mean score (final visit)**	**total TNSS score (baseline)**	**total TNSS score (final visit)**
grass only	7	4	40	21
grass and cat house dust mite	8	5	144	99
cat/house dust mite	7	4	36	22

b)

allergen	**comparison at baseline between placebo group and treatment group p value**	**comparison at final visit between placebo group and treatment group p value**
grass only	0.6030	0.1388
grass and cat/house dust mite	0.3125	0.0093**
cat/house dust mite only	0.6241	0.1443

** statistically significant

placebo group, where the TNSS scores either remained the same or changed by only one score. In the analysis of the treatments only the grass and cat/house dust mite allergen group showed a difference that is statistically different (0.0093**) (**table VIb**). However, a p value of 0.1388 (grass only) and 0.1443 (cat and house dust mite only) was observed between the placebo and treatment group at final visit. Although not significantly different, the p value observed at between the placebo and treatment group at baseline visit were p = 0.6030 and p = 0.6241, respectively (**table VIa**).

Other allergic responses

Analysis of the scores for itchy throat and itchy mouth showed that there was no significant difference between the treatment and placebo groups at the baseline visit for either of these two symptoms. At the final visit symptoms of itchy throat (p = 0.105) and itchy mouth (p = 0.20408) were not significantly reduced by phototherapy (**table VII**). Analysis of the scores for coughing showed that there was no significant difference between the treatment and placebo groups at the baseline visit (p = 0.2301). At the final visit there was a reduction in the total coughing scores for the treatment group which was found to be statistically significant (p = 0.00341**).

Discussion

Allergic rhinitis is the most frequent atopic response which affects potentially 25%-35% of the adult population and this shows an upward trend (20-22). Previous studies reported using controlled conditions showed that persistent allergic rhinitis patients benefited from adding phototherapy to the medical treatment, using combined UVA, UVB, and visible lights (mUV/vis) (23). In these studies, nasal obstruction, sneezing, rhinorrea, and nasal itching showed statistically significant improvement after rhinotherapy at both 1st and 3rd month evaluations for each group, when compared with pretreatment

Table VII *- Total symptom scores and significance value for itchy throat (p value).*

	total score at baseline	**total score at final visit**	**p value**
placebo group	66	60	
treatment group	63	32	0.105

scores (for each symptoms $p < 0.05$). The major goal of the study reported here was to determine if there was an effect of phototherapy on symptoms of allergic rhinitis and other allergic responses. Within the clinical trial, the results showed that rhinophototherapy improved nasal symptoms of allergic rhinitis and other allergic symptoms (coughing), which could potentially also alleviate symptoms. This paper reports on a study which was conducted to assess the ability of a photoperiod device in reducing symptoms associated with allergic rhinitis, which has a high incidence rate amongst the population and has the potential to affect quality of life. Medicines such as steroids and anti-histamines are traditionally prescribed as over the counter medical therapies, but there are many sufferers who do not wish to take medication or for who medication is contraindicated. There are also allergic rhinitis sufferers who wish to reduce the amount of medication that they take, or who find that medication is not sufficient to control their symptoms. In other reported studies, the clinical efficacy of rhinophototherapy (doses of mUV/vis light for 2 weeks) was compared to the antihistamine, fexofenadine hydrochloride. Rhinophototherapy was significantly better than fexofenadine hydrochloride treatment, with respect to the reduction of individual symptom scores for rhinorrhea, nasal obstruction and total nasal scores (24). Phototherapy may be suitable for sufferers in those cases either as a replacement therapy or used alongside traditional medication. The results of the study reported here indicate that this phototherapy device is particularly effective for the nasal symptoms of allergic rhinitis which fall into the mild/moderate range. The nasal symptoms consist of a runny nose, blocked nose, itchy nose and sneezing. Seven participants from the treatment group had no symptoms or markedly reduced symptoms at the end of the study in relation to their TNSS and the six participants from this group who had severe nasal symptoms at the start, had them reduced to moderate or mild at the end of the study. All participants in the treatment group had some reduction in one or more of their nasal symptoms.

The phototherapy device was not shown to be effective for the ocular symptoms, but the effect was statistically significant for coughing. There is an indication that the reduction of nasal symptoms can have a secondary effect of helping to alleviate the symptoms of itchy throat and the need for coughing by reducing excessive mucus production.

This study demonstrates that phototherapy may be an effective method for treating and reducing the effects of symptoms for sufferers of allergic rhinitis particularly those affecting the nose. The device could be used in place of other treatments for some sufferers or as an additional treatment for those who find that traditional medication is not sufficient to control their symptoms or when allergen levels are particularly high (25). In this study, phototherapy was shown to be effective in reducing symptoms attributed to several allergens alone or in combination. This makes it particularly useful in the treatment of allergic rhinitis.

Conflicts of interests

The authors declare that they have no conflict of interest.

Fundings

Advantage West Midlands

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Skin tests and challenge-based drug allergy diagnosis: a retrospective study of patients with confirmed drug allergy

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Key words
drug allergy; drug challenge test; skin tests; predictors; decision tree

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Summary
Objectives. *To describe clinical manifestations and performed diagnostic workup, focusing on drug challenge tests (DCT), in patients with drug allergy.* **Methods.** *Retrospective study including all patients with skin tests (STs) or DCT-based drug allergy diagnosis between 01/2014 - 06/2018 in a Portuguese allergy unit. Data were collected from electronic and paper-based clinical records.* **Results.** *We had 75 drug allergy diagnoses. Most index reactions were mild and ≥ 1 hour after drug intake. Fifty-nine (78%) diagnoses were based on DCTs, all based on multistep protocols with ≥ 3 predicted steps. Only 10% of the DCT were positive during up-dosing; timing and severity of the index reaction predicted DCT interruption during up-dosing.* **Conclusions.** *Most drug allergy diagnoses were based on multistep DCT. The identified predictors of DCT interruption during up-dosing can support the development of more personalized DCTs protocols.*

Introduction

The term "drug allergy" is widely used in a popular sense to encompass both some type A reactions, which are predictable side effects due to the drug's pharmacological action, and type B reactions, which represent true hypersensitivity due to idiosyncratic and individual predisposition (1). However, the definite classification of a drug hypersensitivity reaction (DHR) is important for determining appropriate diagnostic procedures; immunological drug reactions can be divided into two broad types, as recommended by the World Allergy Organization (WAO) (1). These categories are based on the timing of the symptoms' onset: immediate DHRs occur within the first hours of the first administered dose and are usually IgE-mediated, while nonimmediate DHRs occur anytime thereafter; most of these reactions are cell-mediated hypersensitivities and involve several unknown mechanisms, which act simultaneously or even sequentially. However, the precise cut-off to differentiate immediate from nonimmediate reactions is controversial (2-5).
DHRs are often self-reported as "drug allergy" and not confirmed by appropriate assessment. This is a frequent problem in daily clinical practice and has a considerable impact on prescription choices and patient health. In fact, many more patients suspect they have a DHR than can be confirmed, indicating the importance of an accurate diagnosis of DHRs, which will facilitate appropriate treatment options and preventive measures (6). If the reaction is A-type, it is likely that the primary care physician will be able to manage it within the practice, however if it is a B-type reaction, it will require a structured diagnostic process by an allergy specialist (6,7). The diagnostic approach to DHRs may include a detailed clinical history, followed by skin tests (ST), in vitro tests, and drug challenge tests (DCT).

Since clinical history can be unreliable, the sensitivity of in vitro tests may be suboptimal and ST are not feasible nor validated for all drugs, a definitive diagnosis of drug hypersensitivity frequently relies on DCT (8). This test (double blinded, placebo controlled) is widely considered the gold standard for establishing or ruling out drug hypersensitivity. Moreover, it is also of major importance for assessing tolerance to potentially cross-reactive drugs and for providing alternative drugs (8). DCTs involve the controlled administration of a drug under medical surveillance. Therefore, it is a time-consuming and costly exam. A base-case penicillin allergy evaluation including skin testing followed by DCT is estimated to cost $ 220 (9). Evaluation of all Americans who report penicillin allergy would cost over $ 7 billion using this protocol (9-11).

There are a few published protocols for DCTs, but there is lack of information on the best protocol to use with each patient. In clinical practice, the diagnostic approach taken is highly inconsistent despite efforts from the scientific community to create clear-cut algorithms. In most European countries the diagnostic assessment takes place in specialized centers and is adapted depending on the drug involved and the type of allergic reaction suspected (e.g. immediate or non-immediate) (6). Different protocols have been described for immediate and nonimmediate DHRs, with some studies reporting increased diagnostic accuracy when prolonged DCTs are used in individuals with nonimmediate DHRs reactions (12-16). However, even in these nonimmediate DHRs tested with prolonged DCT protocols and having mean reaction delays > 48 hours (17,18), most studies report long first day, office-based, supervised oral DCTs, with multiple increasing doses that could possibly be shortened; this would result in decreased utilization of time and resources. Studies describing diagnostic procedures that confirmed drug allergy and reporting the predictors of DCT positivity during up-dosing are lacking; however, this could give valuable data to inform if shorter protocols for DCT could be safely used, at least in some patients.

With the present study, we aimed to: 1) describe drug allergy manifestations in patients with ST or DCT-based drug allergy diagnosis; 2) describe the diagnostic procedures that were performed to objectively diagnose drug allergy, focusing DCTs; and 3) estimate the proportion of DCTs interrupted before reaching the target cumulative dose, to describe the reacting dose/step and to make an exploratory analysis of the predictors of DCT interruption during up-dosing.

Material and methods

Study design and data collection

This was an observational, retrospective study held in a private allergy unit from Northern Portugal. All patients that had drug allergy confirmed by ST or DCTs between January 2014 and June 2018 were eligible for inclusion. Patients with allergic contact dermatitis to drugs were excluded.

Data were collected from the electronic medical records, the final patient report and the specific DCT paper-forms that are used in the usual clinical practice for data registry; these paper forms include the prespecified protocol, doses administered, the symptoms and objective clinical manifestations arising during DCTs, and DCT outcome. Data was collected considering the variables suggested in the ENDA questionnaire (19).

All patients gave written informed consent to perform the diagnostic procedures. The collected data was anonymized before analysis.

Diagnostic procedures

The drug allergy workup was performed according to the physician's judgment, based on the EAACI guidelines on drug allergy diagnosis (4) and adapted according to the patient's history. The approach to a patient with suspected drug allergy involves a thorough characterization of the index reaction by the physician and, eventually, performing ST (skin prick tests, intradermal tests, patch tests), in vitro tests (e.g. specific IgE), and DCTs.

ST were done and interpreted according to the recommendations from Brockow et al. (20). ST were predominantly performed with drugs that have published validated concentrations (e.g. betalactams). However, in a few patients, especially in those who presented severe immediate index reactions, ST were performed with drugs without fully validated ST concentrations; in these cases, a literature review was performed prior to ST and the tested concentrations were selected according to the best available evidence. In patients with compatible index reaction performing ST with previously published non-irritant drug concentrations (even if not fully validated), especially if the suspected drug was not amenable to DCT, positive ST were considered sufficient to diagnose drug allergy. ST were not routinely performed when the suspected mechanism underlying the index reaction was not suggestive of being amenable to study with ST (e.g. most reactions with NSAIDs) (4).

DCTs were performed using multistep protocols, with administration of progressively higher doses of the suspected or alternative drug. DCT were continued until the therapeutic dose was reached, an objective adverse reaction arisen, or the patient revoked consent for the procedure. In our unit, all DCT are performed under medical supervision in a day ward setting. The usual DCT protocol depends on the drug and can be adapted according to the patient history or symptoms during the challenge; when available, previously published protocols were preferred. All DCT included an extended watching period with length adapted to the severity and time of onset of the index reaction.

Patients with late reactions described as maculopapular exanthems had extended DCTs (at least three days of outpatient

drug intake), according to the findings of some recent studies, both in pediatric (12,13,18,21) and adult (12,14-16) populations. Most patients with late reactions described as maculopapular exanthems (e.g. to betalactams), performed DCT without previous ST (12).
DCTs were considered positive based on the presence of objective signs and reproducibility between DCT and index reaction. When the patient presented no objective signs or nonreproducible minor symptoms, the DCT was considered inconclusive and was not included in this analysis.
Patients with severe cutaneous manifestations (4,22), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), acute generalised exanthematous pustulosis (AGEP), vasculitis or drug reaction with eosinophilia and systemic symptoms (DRESS) were not considered for diagnostic DCT.

Variables and classifications

The up-dosing phase of the DCT was considered completed if the predefined target cumulative dose (at least one therapeutic dose of the specific drug) was reached. When this dose was not attained, the up-dosing phase was considered as interrupted.
Cutaneous drug hypersensitivity reactions were classified according to the EAACI recommendations (22).
The severity of systemic reactions was classified according to the classification proposed by Cox et al. (23), including 5 different grades. This classification system intends to be a common way to describe the severity of systemic allergic reactions, applicable not only to immunotherapy (as the WAO classification system (24)), but also to drug, food or venom allergy. However, it seems primarily designed to classify severity of immediate reactions and does not clearly state that can be applied to delayed DHR. In this study, this classification system was used irrespectively of the time of reaction onset.
The cut-off to define immediate and nonimmediate drug allergy is still controversial: Levine et al. (5) defined immediate reactions as those beginning < 2 hours after drug intake, accelerated between 2 and 48 h, and delayed after 48 hours; Romano et al. (2) considered immediate beginning ≤ 1 hour and non-immediate > 1 hour; other authors (3,4,25) reported that immediate reactions can begin until 6 hours of drug intake and nonimmediate anytime thereafter. To perform exploratory analysis including all these different possible cut-offs, we classified the timing of index and DCT reactions into 5 classes (≤ 1 hour, 1 to 2 hours, 2 to 6 hours, 6 to 48 hours, and > 48 hours).

Statistical analysis

Data was analysed by patient and by drug allergy diagnosed. In patients with more than one reaction to the same drug, the most recent was included in the descriptions.
Categorical data was described with absolute and relative frequencies and continuous variables (age) with mean and standard deviation (SD). Pearson chi-square was used for comparison of proportions when all categories had n > 5; in very small groups (at least one category with n ≤ 5) a 2-sided Fisher's exact test was used. Variables organized as trend were analysed with linear-by-linear association chi-square. Comparisons of continuous variables, due to the small sample size and variable distribution, were performed using non-parametric tests, including Kruskal-Wallis (for k independent samples) and Mann Whitney U (for 2 independent samples).
Agreement between categorical variables was assessed with Cohen's kappa (K). The K value was interpreted as follows (26): < 0.20 poor; 0.20 to 0.40 fair; 0.41 to 0.60 moderate; 0.61 to 0.80 good; 0.81 to 1.00 very good.
We used CART (classification and regression tree) analysis to identify the most important predictors of DCT outcome during up-dosing. CART is a nonparametric supervised classification method that is intended as an exploratory tool to discover homogeneous subgroups within the data; it is appealing because of the apparent closeness to the human reasoning processes, presenting data in easy to interpret tree models (27). We performed CART analysis using Gini impurity index to grow the trees and the cost-complexity pruning algorithm to generate a simpler tree. The chosen result is within one standard error of the tree with best error estimate, favouring trees with minimum costs. The predictors included in CART were selected based on an unadjusted analysis that identified the variables significantly associated with DCT outcome during up-dosing. The variable importance was given by Gini index, where the highest value is 100%. Variables that did not contribute significantly to the model were automatically removed. CART analysis was performed with Salford Predictive Modeler® version 8.2 (Salford Systems, San Diego, USA). All other data analyses were performed with IBM SPSS® version 25 (IBM Corporation, Armonk, USA). P-values < 0.05 were defined as statistically significant.

Results

We performed 589 drug allergy workups, including 528 DCT and 179 sessions of ST, in 496 patients. During the study period, we had 72 (15%) patients with drug allergy diagnosis confirmed with ST or DCT. Seventy one percent (n = 51) were female with a mean (SD) age of 34 (21) years; 21 (29%) were < 18 years.
Two patients had confirmed allergy to more than one drug (maximum 3), totalling 75 drug allergies diagnosed.

Characteristics of the index reactions

The characteristics of the index reactions are described in **table I**. About half of the reactions were caused by betalactams (45%,

Table I *- Characteristics of the index reactions, including stratification by the diagnostic procedure that confirmed drug allergy. When more than one reaction was reported for the same drug, the most recent was included. ST were performed in 31 (41%) of the included reactions.*

	Total		**Procedure that confirmed diagnosis**				
			ST[1]			**oral DCT**	
	(n = 75)		**done (n = 31)**	**positive (n = 16)**		**(n = 59)**	
	n	(%)	n	n	%	n	%
Drug involved / suspected							
penicillins	34	(45)	15	7	(44)	27	(46)
other antibiotics[2]	12	(16)	7	5	(31)	7	(12)
paracetamol	4	(5)	2	0	(0)	4	(7)
NSAIDs	19	(25)	1	0	(0)	19	(32)
other drugs[3]	6	(8)	6	4	(25)	2	(3)
Time since last reaction							
≤ 12 months	43	(67)	20	11	(73)	32	(66)
12 to 36 months	7	(11)	2	2	(13)	5	(10)
36 to 120 months	7	(11)	3	1	(7)	6	(12)
≥ 120 months	7	(11)	5	1	(7)	6	(12)
Symptoms							
Cutaneous	71	(97)	30	15	(94)	56	(98)
urticaria[4] and/or angioedema	53	(73)	23	14	(88)	39	(68)
maculopapular exanthem	18	(25)	6	0	(0)	18	(32)
fixed drug eruption	2	(3)	0	0	(0)	2	(4)
Respiratory	19	(26)	10	6	(40)	13	(23)
lower airways	18	(25)	9	6	(43)	12	(21)
upper airways (including laryngeal)	7	(10)	2	0	(0)	7	(12)
Cardiovascular	8	(11)	7	6	(38)	2	(4)
hypotension / collapse	8	(11)	7	6	(38)	2	(4)
Gastrointestinal	4	(56)	4	0	(0)	4	(7)
Other / unspecific	7	(10)	2	2	(13)	5	(9)
Severity, considering ref. (23)							
grade 1	48	(68)	16	7	(44)	41	(75)
grade 2	3	(4)	1	0	(0)	3	(6)
grade 3	12	(17)	7	3	(19)	9	(16)
grade 4	0	(0)	0	0	(0)	0	(0)
grade 5	8	(11)	7	6	(38)	2	(4)
Time of reaction onset							
≤ 1 hour	23	(33)	15	14	(93)	9	(16)
1 - 2 hours	5	(7)	3	1	(7)	4	(7)
2 - 6 hours	5	(7)	2	0	(0)	5	(9)
6 - 48 hours	12	(17)	3	0	(0)	12	(22)
> 48 hours	25	(36)	6	0	(0)	25	(46)
Previous reaction with same / related drug	33	(46)	11	3	(19)	30	(54)

[1]Includes skin prick tests and intradermal tests; [2]Including cephalosporins, ciprofloxacin, cotrimoxazole, levofloxacin, minocycline, nitrofurantoin, vancomycin; [3]Including albendazole, atropine, betamethasone, cisatracurium, influenza vaccine, patent blue; [4]Includes urticaria and/or erythema-warmth and/or pruritus, other than localized at the injection site (23). NSAID, nonsteroidal anti-inflammatory drug.

n = 34 penicillins, and 5%, n = 4 cephalosporins). Two thirds of the patients had the last reaction to the culprit drug in the past 12 months and about half reported at least another previous reaction with the same or a related drug. Almost all patients reported cutaneous symptoms, one quarter respiratory involvement, and one tenth had hypotension and/or collapse; sixty-one percent (n = 46) of the reactions presented with exclusive cutaneous symptoms. Most reactions presented grade 1 severity; eight (11%) were grade 5. One third of the index reactions occurred in the first hour after drug intake. Two patients had no information regarding symptoms and timing of the index reaction: one child with asthma that had confirmed anaphylaxis to paracetamol and tested ibuprofen to exclude possible cross-reactivity; another patient did not remember any characteristic of the previous reaction.

Description of the diagnostic procedures performed

Two (3%) drug allergies were confirmed by skin prick tests and 14 (19%) by intradermal tests. Fifty-nine (78%) diagnoses were made by oral DCT. None of these patients performed patch tests. Characteristics of the index reactions stratified by the diagnostic procedure that confirmed drug allergy are presented in **table I**. More than half of the ST were performed in patients with very immediate (< 1 hour) index reactions and almost all were positive. No patient with a reaction beginning more than 2 hours after drug intake had positive ST. Otherwise, 75% of the patients with grade 5 reactions had diagnosis confirmed by positive ST.

Fifty-four (92%) DCTs were performed with diagnostic intent. The other five DCTs intended to find a suitable alternative within the class of nonsteroidal anti-inflammatory drugs (NSAID). Two of these patients presented chronic urticaria with multiple NSAID intolerance, including to selective COX-2 inhibitors (meloxicam and etoricoxib); as both of them had indication to take NSAID due to comorbid diseases, we decided to perform the DCT under preventive treatment with anti-histamine ± montelukast to increase the odds of finding a suitable alternative strategy that could give an answer to their need of NSAID intake. Nevertheless, even with this preventive strategy, the DCT were positive.

All DCTs were performed using multistep protocols, with at least three predicted steps (maximum 7 steps). The number of predicted steps was not significantly and independently associated with symptoms, severity or time of onset of the index reaction, but was significantly associated with the drug tested ($p = 0.033$). DCTs with penicillins had the highest proportion of procedures with at least 6 predicted steps (65% vs. 29% with other antibiotics, vs. 26% with NSAIDs vs. 0% with paracetamol and other drugs; $p = 0.013$).

Description of DCTs results

Six (10%) DCTs were considered positive and interrupted during the up-dosing phase. In these DCTs, the reaction occurred at 4 to 45% of the predicted cumulative dose; two patients had positive DCT with less than 10% of the predicted dose. The proportion of missed steps ranged from 17 to 67% of the predicted. Ninety percent (n = 53) of the DCTs had the up-dosing phase completed. Thirteen (22%) were positive during the watching period and 40 (68%) after day ward discharge (at least 5 hours after the last supervised dose intake). Thirty-four patients had extended DCT, maintaining drug intake in the days after discharge; the median time to reaction was 5.5 days (maximum 11 days). About two thirds (n = 37) of the DCT reactions occurred within the same time period reported for the index. However, 15 (27%) were faster during DCT. Five (9%) reactions with index onset between 2 and 48 hours occurred within the first hour of the last drug intake during DCT. No reaction with index onset > 48 hours had symptoms within the first 2 hours.

A description of the symptoms presented during the DCT is presented in **table II**. Almost all patients presented cutaneous symptoms, with a complete agreement with the index reaction. However, the specific type of cutaneous reaction had low agreement (except for fixed drug eruption). Cardiovascular symptoms were present in only one patient.

Seventy-two percent (n = 41) of the DCT reactions were grade 1; only one patient had a grade 5 reaction. Eighty percent (n = 44) of the DCT reactions were of similar severity grade as index reactions. Six (11%) had higher severity during DCT; the largest variation (from a grade 1 index reaction to a grade 4 DCT reaction) occurred in one patient that reported a mild cutaneous reaction to penicillin more than 10 years before the DCT.

Predictors of DCT outcome during up-dosing - exploratory analyses

The characteristics of patients that interrupted DCT are presented in **table III**.

DCTs that were interrupted during up-dosing were performed with NSAIDs, paracetamol and levofloxacin; no penicillin DCT was positive/interrupted during up-dosing. All patients that interrupted DCT before reaching the cumulative dose reported lower airways symptoms ($p < 0.001$) and very immediate index reactions ($p < 0.001$) with at least grade 3 severity.

No significant associations were found between DCT outcome during up-dosing and gender ($p = 0.658$), age group ($p = 0.653$), time since last reaction ($p = 0.682$), another or index reaction with cutaneous (0.754), cardiovascular (0.169) or gastrointestinal (0.315) symptoms.

All variables identified as being significantly associated with DCT outcome (**table III**) were included in the CART analysis

Table II - *Symptoms present at the DCT and comparison with the reported index reactions. Two patients had missing information regarding the index reaction.*

	Total (n = 59)		Comparison with index reaction								
			concordant				discordant				kappa
			present		absent		present in index		present in dct		
	n	(%)	n	%	n	%	n	%	n	%	
Cutaneous	58	(98)	56	(98)	1	(2)	0	(0)	0	(0)	1.000
urticaria[1] and/or angioedema	36	(61)	20	(35)	15	(26)	8	(14)	14	(25)	0.231
maculopapular exanthem	26	(44)	16	(28)	29	(51)	2	(4)	10	(18)	0.565
fixed drug eruption	2	(3)	2	(4)	55	(97)	0	(0)	0	(0)	1.000
Respiratory	16	(27)	11	(19)	41	(72)	2	(4)	3	(5)	0.757
lower airways	6	(10)	5	(9)	45	(79)	7	(12)	0	(0)	0.530
upper airways (including laryngeal)	12	(20)	5	(9)	45	(79)	2	(4)	5	(9)	0.519
Cardiovascular	1	(2)	0	(0)	54	(95)	2	(4)	1	(2)	0.024
Gastrointestinal	1	(1)	1	(2)	53	(93)	3	(5)	0	(0)	0.383

[1]Includes urticaria and/or erythema-warmth and/or pruritus, other than localized at the injection site (23).

Table III - *Patients' characteristics according to the DCT outcome during the up-dosing phase (interrupted vs. completed). Two patients had no information regarding the symptoms of index reaction.*

	DCT outcome during up-dosing[1]				
	interrupted (n = 6)		completed (n = 53)		p-value
	n	%	n	%	
Gender, female	4	(67)	39	(74)	0.658
Age group, < 18 years old	1	(17)	19	(36)	0.653
Drug involved					**0.026**
penicillins	0	(0)	27	(51)	
other antibiotics[2]	1	(17)	6	(11)	
paracetamol	2	(33)	3	(4)	
NSAIDs	3	(50)	16	(32)	
other drugs[3]	0	(0)	2	(4)	
Time since last reaction					0.682
≤ 12 months	3	(60)	29	(66)	
12 to 36 months	1	(20)	5	(9)	
36 to 120 months	1	(20)	5	(11)	
≥ 120 months	0	(0)	6	(14)	
Symptoms, index reaction					
Cutaneous	5	(100)	51	(98)	0.754
urticaria[4] and/or angioedema	5	(100)	34	(65)	0.112

Table III - *(continued)*

	DCT outcome during up-dosing[1]				
	interrupted (n = 6)		completed (n = 53)		p-value
maculopapular exanthem	0	(0)	18	(35)	0.168
fixed drug eruption	0	(0)	2	(4)	1.000
Respiratory	5	(100)	8	(15)	**< 0.001**
lower airways	5	(100)	7	(14)	**< 0.001**
upper airways (including laryngeal)	2	(40)	5	(10)	0.109
Cardiovascular	1	(20)	1	(2)	0.169
hypotension / collapse	1	(20)	1	(2)	0.169
gastrointestinal	1	(20)	3	(6)	0.315
Other / unspecific	1	(20)	4	(8)	0.379
Severity index, considering ref. (23)					**< 0.001**
grade 1	0	(0)	41	(82)	
grade 2	0	(0)	3	(6)	
grade 3	4	(80)	5	(10)	
grade 4	0	(0)	0	(0)	
grade 5	1	(20)	1	(2)	
Time of index reaction onset					**< 0.001**
≤ 1 hour	6	(100)	3	(6)	
1 - 2 hours	0	(0)	4	(8)	
2 - 6 hours	0	(0)	5	(10)	
6 - 48 hours	0	(0)	12	(25)	
> 48 hours	0	(0)	25	(51)	
Another previous reaction with same / related drug	4	(80)	26	(51)	0.358

[1]The up-dosing phase of the DCT was considered completed if the target cumulative dose was reached; [2]Including cephalosporins, ciprofloxacin, cotrimoxazole, levofloxacin, minocycline, nitrofurantoin, vancomycin; [3]Including albendazole, atropine, betamethasone, cisatracurium, influenza vaccine, patent blue; [4]Includes urticaria and/or erythema-warmth and/or pruritus, other than localized at the injection site (23). NSAID, nonsteroidal anti-inflammatory drug.

as possible predictors. CART identified the timing (≤ 1hour vs. > 1 hour) and severity (grade ≤ 2 vs. grade ≥ 3) of the index reaction as the most important predictors of DCT outcome during up-dosing (**figure 1**). The final decision tree presented a classification accuracy of 98.3%, with only one patient misclassified; all patients that had the DCT interrupted during up-dosing were correctly identified.

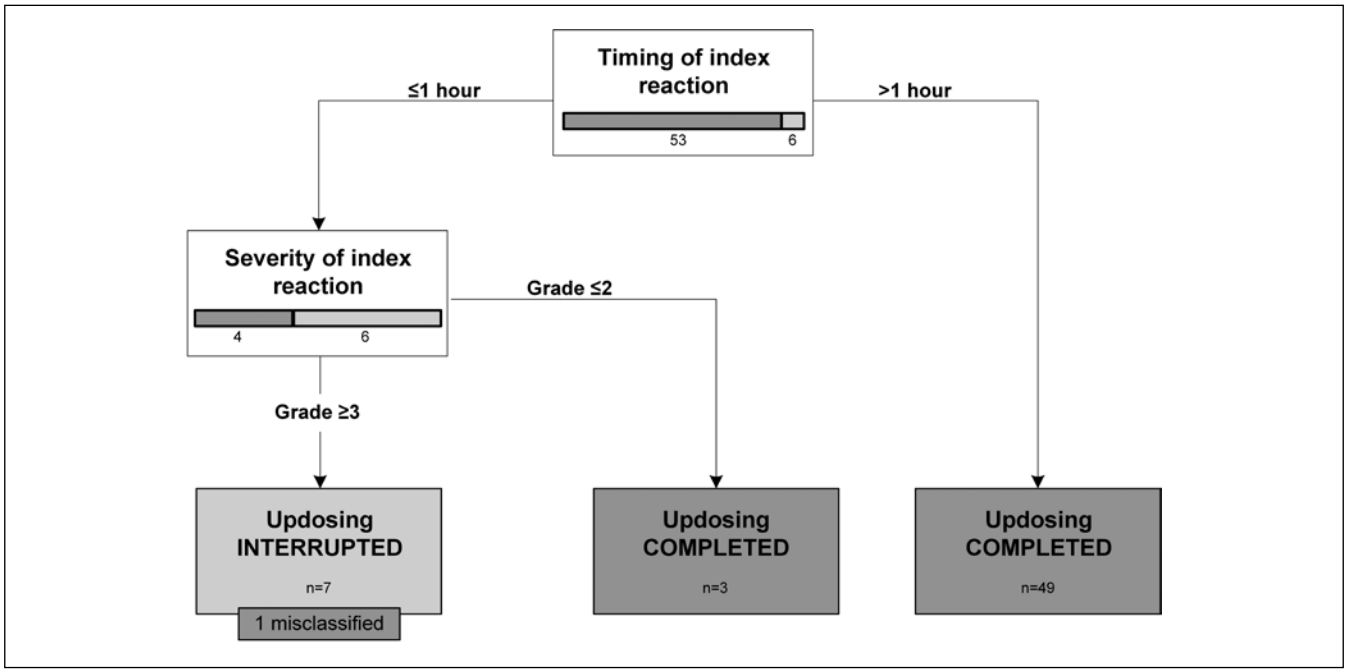
Discussion

In this study, the prevalence of ST or DCT-based allergy diagnosis was 15%. Most index reactions were mild, presenting only cutaneous symptoms and beginning more than one hour after drug intake. Seventy-eight percent of the diagnoses were based on DCT. All DCTs were performed using multistep protocols with at least three predicted steps; the number of DCT steps was associated with the drug tested but not with symptoms, severity or time of index reaction onset. Although most DCT reactions were mild, 11% were more severe than reported for the index reaction. Only 10% of the DCTs were considered positive and interrupted during up-dosing; in about 70% of the DCTs the reaction begun at least 5 hours after the last supervised drug intake. The timing (≤ 1 hour vs. > 1 hour) and severity (grade ≤ 2 vs. grade ≥ 3) of the index reaction were the most important predictors of DCT outcome during up-dosing.

Strengths and limitations

To our knowledge, this is the first study specifically assessing predictors of DCT outcome during the up-dosing phase. Having a good prediction rule for the outcome of the supervised up-dosing phase of DCT can support the development of short-

Figure 1 *- Decision tree for DCT outcome during up-dosing (interrupted vs. completed).*



er and personalized DCT protocols in patients with low risk of reaction, decreasing the cost and time of these diagnostic procedures without increasing the risk of higher severity DCT reactions. However, this study is limited by the retrospective nature, with data collected from electronic and paper-based data recording systems. Moreover, the small sample size, especially in the analyses using comparisons between groups of DCT outcome and CART, makes these results only exploratory and in need of careful interpretation. The selection of patients with confirmed drug allergy, which would not be possible before diagnostic assessment and is inverted comparing to real-life, makes this study less directly applicable to clinical practice. However, we considered that it might be more effective to describe and evaluate predictors of DCT outcome in patients that were at "true" risk of interrupting DCT due to reaction. The inclusion of all patients, irrespective of the results of diagnostic assessments, although closer to the usual diagnostic reasoning, would increase the noise and decrease the focus in those patients. Nevertheless, before clinical use, these results need validation in a more comprehensive sample including patients with and without confirmed drug allergy.

The inclusion of patients presenting mostly mild reactions and predominantly with betalactams might also influence our results. Yet, these were the available patients in our centre and we believe they represent the usual clinical practice in most allergy units.

Interpretation of study findings and comparison with the literature

Previous studies, in various settings and populations, with diverse drugs and based on different diagnostic approaches, showed a prevalence of confirmed drug allergy in subjects with suspected drug reactions ranging from 3 to 27% (28-31). Our prevalence is higher than those reported in general settings (around 6%) (28,31), but is in line with a few studies held in specialized allergy units (30).

In our study, most patients presented the last reaction to the culprit drug within the previous 12 months. This puts most patients within the best time interval to perform the diagnostic assessment of suspected drug allergies. Indeed, a loss of sensitivity to drugs over time has been reported for IgE-mediated reactions (32), and after a time-interval of more than 6-12 months, some drug tests may already give negative results. Moreover, when the time interval between the reaction and the allergy assessment is longer, history is often less reliable and there is a lack of accurate information: the chronology is imprecise, the clinical manifestations are heterogeneous, making drug causality assessment more difficult to ascertain (6,33). The short time interval between the index reaction and diagnostic assessment might have contributed to the low number of patients with missing data regarding the characteristics of the previous reactions. However, the agreement between the characteristics of the index and DCT reactions was only fair, especially regarding cardiovascular, gastrointestinal and

specific cutaneous symptoms, suggesting that symptoms may be interpreted differently at different times/by different assessors or that DCT reaction might vary from the index reaction, even when it is recent; these findings are in line with another recent study (34). Nevertheless, most patients presented DCT reactions of similar severity as the index.

There are several difficulties regarding the choice of the best diagnostic approach to a specific patient, namely when to perform ST and which DCT protocol should be used. One of the controversies is related to the cut-off that should be used to differentiate immediate from nonimmediate reactions (2-5). In our study we chose to assess drug allergy diagnoses without *a priori* stratification by timing of the index reaction. This allowed us to explore the best cut-off to identify patients at higher risk of early DCT reaction (≤ 1 hour vs. > 1 hour), which, in our study, follows the classification proposed by Romano et al. (2).

In the past decade, several studies have evaluated prolonged DCTs to better detect nonimmediate DHRs with penicillin antibiotics (12-18). A recent study used a similar approach also in patients with non-severe immediate amoxicillin reactions beginning with an office-based supervised 3-steps DCT followed by a 4-day DCT; this study showed that this is a safe and effective way to rule out non-severe immediate and nonimmediate amoxicillin allergy, and ensures better compliance with future penicillin use (35). In that study, only 2.3% among the 130 patients who underwent a DCT presented a reaction on their initial visit (even if 20% had a suspected immediate reaction based on the clinical history) and all the remaining had to undergo a further ambulatory course of antibiotic continued at home (35). However, even in these studies, the diagnostic approach included a long, ≥ 3 steps, office-based initial DCT. Only a few studies assessed the safety of shorter (1 or 2-step) DCT. Iammatteo M et al. (36) performed a retrospective study of 497 one or two-step test doses and compared the outcomes with those of multistep challenges. They included patients tested with several drug classes and found that one or two-step DCT were safe and that multistep challenges did not confer added safety. Mawhirt SL et al. (34) also reported that full dose challenges presented similar safety to multistep DCT in patients with immediate reactions to antibiotics. Our results, with most reactions occurring after the target dose was reached, irrespective of the tested drug, support that these shorter DCT/test doses might be effective and safe way to diagnose drug allergy, at least in some patients.

In fact, DCT are long and expensive (9-11) diagnostic procedures that would benefit from a better patient stratification through the identification of predictors of early DCT reaction. We found timing and severity of index reactions as the best predictors of DCT outcome during up-dosing. Indeed, timing and severity of the index reaction are traditionally used to stratify diagnostic procedures to perform in patients with suspected drug allergy (3,4,10). However, besides controversial classifications, there are no clearly defined cut-offs and practical decision rules to apply when selecting the diagnostic approach to follow in a specific patient. Our CART analysis, although exploratory, allowed the development of a decision tree that could be used to correctly identify patients that had the DCT interrupted. Our results suggest that, in most patients, DCT protocol could be more adapted to the patient and index reaction characteristics and less dependent on the drug. However, further studies comparing multistep with full dose or two-steps DCT protocols are warranted. The predictors of DCT outcome during up-dosing, found in our exploratory analysis, should be further tested in a different sample and validated into a prospective comparative study in real-life conditions.

Conclusions

Most drug allergy diagnoses were based on drug challenges, performed with multistep protocols dependent on the culprit drug. Only one tenth of the challenge reactions occurred during the up-dosing phase. The predictors of DCT interruption during up-dosing identified in the CART analysis can support the development of more personalized DCTs protocols but need further research before being applied into clinical practice.

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Conflict of interests

The authors declare that they have no conflict of interest.

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Subcutaneous immunotherapy with aeroallergens - evaluation of adherence in real life

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Key words

allergy; allergic respiratory diseases; allergen immunotherapy; subcutaneous immunotherapy; adherence

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Summary

Introduction. *Adherence in allergen immunotherapy is crucial for its efficacy. At least 3 years of treatment are recommended for achieving a long-term modifying effect.* **Objectives**. *To assess patient's adherence and to identify determinant factors for allergen subcutaneous immunotherapy (SCIT) suspension in patients with respiratory allergy.* **Methods**. *Retrospective analysis of the medical record of patients submitted to SCIT between January 2013 and December 2016 in our Department.* **Results**. *323 patients were included: 52% female; mean age 30 ± 13 years; average treatment time 19 ± 13 months. 52 patients (16%) stopped SCIT: 54% female; mean age 30 ± 9 years; average treatment time 12 ± 6 months; 67% dropped the treatment during the 1st year, 27% in the 2nd and 6% during the 3rd year of treatment. Adherence rate determined was 77%. The most frequent reasons for withdrawal were due to economic reasons (47.9%), followed by patients' perception of no clinical improvement (23%) and change to sublingual immunotherapy (11.6%).* **Conclusions.** *Adherence rate in our study was 77%. Economic reasons were the main cause of abandonment in the first year, while the perception of non-improvement was the main reason for abandonment in subsequent years. Adequate information on SCIT prescribing and rigorous monitoring of patients during the treatment can improve adherence.*

Introduction

Allergic respiratory diseases, namely rhinitis and asthma, are a major public health problem. Asthma affects an estimated 300 million individuals (1) and allergic rhinitis affects 10 to 40% of the population worldwide (2). These diseases are known to reduce the overall quality of life as well as to increase school and work absenteeism and medical costs (2). Therefore, the correct treatment with adequate control of these diseases is very important.

The key points of the treatment of allergic respiratory diseases are patient education, allergen avoidance and pharmacological therapy (1,2). Allergen immunotherapy has shown to modify the natural history of allergic disease, maintaining beneficial effects even after its cessation and the possibility to prevent the onset of asthma in patients with rhinitis or the appearance of new sensitizations (3). Subcutaneous administration is the main form of immunotherapy with aeroallergens because of its higher effectiveness (4). However, this treatment also has disadvantages: the administration can be painful and it is associated with a higher risk of systemic reactions. Moreover, it is a time-consuming procedure, due to the need for supervised administration by a trained health care professional in a setting with conditions for treating systemic reactions.

Adherence represents the most critical issue and it is essential for achieving good results. Poor adherence to immunotherapy leads to a decrease in treatment benefits that can potentially lead to an increase of morbidity (5). A minimum duration of 3-years of subcutaneous immunotherapy (SCIT), with an optimized

dosing schedule, is required to achieve an adequate clinical and immunological response and long-term efficacy (6).
Adherence can be divided in three different stages: initiation (acceptance), implementation (compliance) and persistence (5). In this study we defined adherence as the accomplishment of at least three complete years of SCIT. Although there is no consensus regarding what an acceptable adherence rate is, most researchers consider an adherence rate greater than 80% to be adequate (5,7). In clinical trials the reported adherence rate is around 80-90% and it is more variable in real-world studies, ranging from 23-88% in adults and 16-89% in children (5).
The reasons for a poor adherence to SCIT may be related to the patient, disease, treatment or healthcare system (5). The identification of these factors can increase the success of immunotherapy. The most common factors associated with a poor adherence to SCIT are: the patient´s knowledge of his/her disease and treatment conditions and benefits, route of administration, treatment inconvenience and costs and side effects (5).
The aim of this study was to evaluate SCIT adherence in patients with allergic rhinitis and/or asthma and to determine the factors that affect adherence to in real-life conditions.

Material and methods

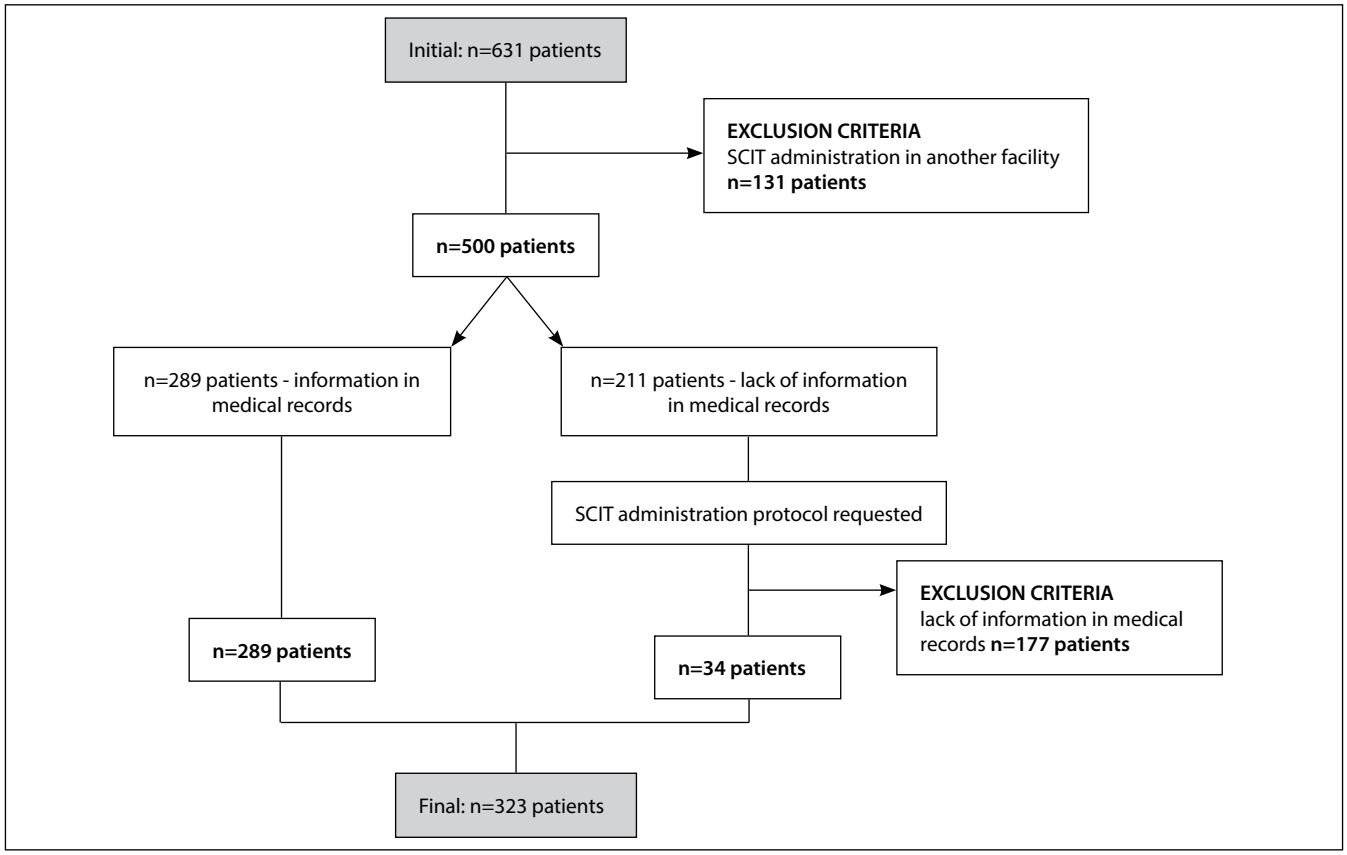
Population and study design

A retrospective analysis of the medical and nursing records of 631 patients submitted to SCIT between January 2013 and December 2016 in our Immunotherapy Center, Allergy and Clinical Immunology Outpatient Clinic of Hospital de Santa Maria, Centro Hospitalar Universitário de Lisboa Norte, was performed. Patients' age, gender, allergic disease diagnosis (rhinitis and/or asthma; eczema; conjunctivitis; food allergy), SCIT composition, date of initiation and SCIT administration schedule were registered and evaluated. The reasons to stop SCIT were also analyzed and evaluated. Switching to sublingual route of immunotherapy was considered a reason of SCIT dropout, once the aim of this study was to specifically evaluate adherence to the subcutaneous route and determine the factors that affect it.
Patients who lacked clinical information about SCIT composition or administration (n = 211), were contacted by letter requesting SCIT administration protocol information**,** with very poor response (response rate 16%). Patients that sent the SCIT administration protocol and had dropout SCIT were contacted again, by call and were asked about dropout reasons.
Patients were excluded from the study if SCIT was administered in another facility (n = 131) or if there was missing information in their medical records concerning SCIT administration, namely SCIT composition, date of initiation and SCIT administration schedule (n = 177) (**figure 1**). This lack of parameters is due to absence of electronic clinical records before 2012/2013 and the impossibility to get access to written medical information.
The diagnosis and treatment of allergic rhinitis and asthma were appropriate according to current guidelines, Allergic Rhinitis and its Impact on Asthma (ARIA) (8) and Global Initiative for Asthma (GINA) (1). Skin prick tests with Roxall® extracts and/or serum specific IgE tests using ImmunoCAP system® (TermoFisher scientific; Uppsala, Sweden) were conducted. All patients had positive skin prick tests and/or specific IgE tests > 0.70 kU/L, and a correlation between these results and their symptoms was found. SCIT was initiated in patients with allergic symptoms despite being under medical treatment. SCIT was chosen considering the results of skin prick tests and/or specific IgE tests to house dust mites, storage mites, pollens (grass, *Parietaria*, olive tree and *Artemisia*) and cat epithelium or extract associations (mites and pollens). The route of therapy (subcutaneous) was prescribed taking into consideration the patient's preference, allergic symptoms and personal concerns.
A written informed consent was obtained from all patients and/or their legal representatives before initiating SCIT.
The maintenance dose was administered at 4-6-week intervals over a period of 3 to 5 years. All injections were administered by trained nurses with supervision of the allergist in the Immunotherapy Center, equipped with material for treating systemic reactions. All patients were evaluated before and 30 minutes after the SCIT administration.
Adherence was determined as the accomplishment of three years of SCIT. The patients who dropped SCIT before this time were considered as non-adherent; the patients that continued the treatment were considered as adherent. To calculate adherence rate, only patients who started SCIT in 2013 were considered in order to have completed the recommended three years of treatment, since it is the minimum to be considered compliant.
Data were anonymized, and their confidentiality guaranteed, and this study protocol was approved by the Ethical Board of Centro Hospitalar Universitário de Lisboa Norte.

Descriptive and statistical analysis

As previously mentioned, the main objective of this study was to assess and identify the main causes behind treatment discontinuation. According to our objectives, we analyzed the group of patients who stopped SCIT and evaluated the causes that contributed to the suspension of SCIT using descriptive statistics. For the descriptive analysis, categorical variables were given as numbers and percentages, and continuous variables were presented using mean, standard deviation, median, interquartile range (IQR) and minimum and maximum values.
Statistical analyses were performed using version 24 of SPSS software for Windows (SPSS Inc., Chicago, Ill). Mann-Whitney U and Kruskal-Wallis tests were used to compare differ-

***Figure 1** - Study design and flow chart.*



ences between groups and p value < 0.05 was considered statistically significant.

Results

From a total of 631 patients under SCIT during the study period, 323 patients met the inclusion criteria and 308 were excluded due to data unavailability.

According to the demographic data (**table I**), there was a predominance of female gender (167, 52%), mean age of the patients was 30 ± 13 years (minimum 7, maximum 65, median 27). The age group of 18 to 30 years was the most prevalent with 45% (n = 145) and the group older than 50 years was the least prevalent with 7.1% (n = 23).

Regarding the allergen used in SCIT, we observed a predominance of mite allergen (233, 72%). More information about SCIT composition is detailed in **table I**.

The diagnosis of patients submitted to SCIT was also evaluated and is provided in **table I**. All patients had allergic respiratory disease, with rhinitis being the most frequent diagnosis (313, 97%) followed by asthma (145, 45%), about 40% of patients had concomitant asthma and rhinitis. The average treatment duration was 19 ± 13 months (maximum 58 months; minimum 1 month). Most patients (70.8%) were in the first 2 years of SCIT and 17.7% completed at least 3 years of treatment. We also evaluated the number of patients by year of treatment: first year 132, 40.8%; second year 97, 30%; third year 37, 11.5%; fourth year 38, 11.8%; fifth year 19, 5.9%.

When comparing the patients who dropped SCIT without medical indication with those who completed the treatment (i.e. adherent group), no statistical differences were found regarding age, gender, clinical diagnosis and allergen extract (**table I**). **Table I** shows the clinical and demographic comparison between the 2 groups (adherent and non-adherent patients).

Adherence was determined at the end of 3 years of SCIT treatment. Fifty-two patients (16%) stopped SCIT without medical indication before the recommended time. In the group of patients who abandoned SCIT (i.e. non-adherent patients), there was a slight predominance of female gender (28, 54%), mean age 30 ± 9 years (minimum 14, maximum 48, median 28).

Table I *- Demographic and clinical data of the patients under subcutaneous immunotherapy.*

Variables	Patients			p-value
	total (n = 323; 100%)	adherent (n = 271; 84%)	non-adherent (n = 52; 16%)	
Age (median; IQR) years	27; 21	26; 20	28; 19	0.073
Age groups				
7 - 17 n (%)	53 (16.4)	49 (18)	4 (7.7)	
18 - 30 n (%)	145 (45)	117 (43)	28 (54)	
31 - 50 n (%)	102 (31.5)	90 (33)	12 (23)	
51 - 65 n (%)	23 (7.1)	15 (6)	8 (15.3)	
Gender				0.525
female n (%)	167 (52)	139 (51)	28 (54)	
male n (%)	156 (48)	132 (49)	24 (46)	
Clinical diagnosis				0.449
rhinitis n (%)	313 (97)	263 (97)	50 (96)	
asthma n (%)	145 (45)	121 (44)	24 (46)	
rhinitis and asthma n (%)	129 (40)	100 (37)	17 (33)	
conjunctivitis n (%)	92 (28.5)	70 (25)	13 (25)	
eczema n (%)	52 (16)	40 (15)	12 (23)	
food allergy n (%)	30 (9)	23 (8)	7 (13)	
Type of allergen extract				0.423
Dermatophagoides (*pteronyssinus* and/or *farinae*) n (%)	172 (53.4)	148 (54.5)	24 (46.2)	
Dermatophagoides + another mite n (%)	41 (12.7)	33 (12.2)	8 (15.4)	
storage mites n (%)	7 (2.2)	4 (1.5)	3 (5.8)	
Dermatophagoides + pollen n (%)	13 (4)	10 (3.7)	3 (5.8)	
grass pollen n (%)	66 (20.4)	56 (20.7)	10 (19.2)	
Parietaria n (%)	10 (3.1)	8 (3)	2 (3.8)	
grass pollen + olive tree n (%) grass pollen + *Parietaria*	5 (1.5)	5 (1.8)	0 (0)	
n (%)	4 (1.2)	3 (1.1)	1 (1.9)	
grass pollen + *Artemisia* n (%)	2 (0.6)	1 (0.4)	1 (1.9)	
olive tree n (%)	2 (0.6)	2 (0.7)	0 (0)	
cat epithelium n (%)	1 (0.3)	1 (0.4)	0 (0)	

The most prevalent age group was from 18 to 30 years old (54%) and the least prevalent age group was from 7 to 17 years old (7.7%). Regarding the immunotherapy composition, SCIT suspension for mites was predominant (73%, 16.3% of total SCIT for mites) followed by pollens (32.6%, 13.6% of total SCIT for pollens). The average treatment duration was 12 ± 6 months (maximum 27 months; minimum 4 months).

In order to calculate adherence rate, only patients who started SCIT in 2013 were considered in order to have completed the recommended three years of treatment, since it is the minimum to be considered compliant. Fifty-seven patients started SCIT in this year and 13 stopped it before completing 3 years of treatment, corresponding to an adherence rate of 77%.

Most patients (67%) abandoned SCIT during the first year, 27% in the second and 6% during the third year of treatment. The main reasons for abandoning SCIT without medical indication are presented in **table II**.

Economic reasons were the most frequent factor reported, accounting for almost half of the treatment abandonment (47.9%). Twenty-three percent referred the absence of clinical improvement and around 12% switched to sublingual immunotherapy. Personal issues such as relocation, support to family and professional reasons resulted in 7.7% of suspensions; adverse reactions, namely large recurrent local reactions motivated 3.9% of the SCIT suspension. Two patients (3.9%) stopped SCIT because they were diagnosed with other medical conditions (neoplasm). Pregnancy was the reason behind the withdrawal of 2% of patients; in this case, SCIT abandonment was a patient's choice.

When analyzed the main causes by year, results have shown that the most frequent cause of suspension in the first year was due to economic reasons (21/35, 60%), and the perception of no improvement was the most frequent reason in the following years (7/17, 41%).

Table II *- Reasons for subcutaneous immunotherapy withdrawal.*

Reasons for SCIT withdrawal	**Non-adherent patients (n = 52)**			
	1st year n = 35	2nd year n = 14	3rd year n = 3	total n (%)
economic reasons	21	4	0	25 (47.9)
no clinical improvement	5	7	0	12 (23)
switch to sublingual immunotherapy	4	2	0	6 (11.6)
personal issues	2	0	2	4 (7.7)
adverse reactions	2	0	0	2 (3.9)
medical illness	0	1	1	2 (3.9)
pregnancy	1	0	0	1 (2)

Discussion

In our real-life study, the adherence rate was 77%. In total, 52 (16%) patients dropped out: 35 patients (67.3%) in year 1, 14 (27%) in year 2, and 3 (5.7%) in year 3. Reviewing the literature, we find that reported SCIT adherence rates are very variable, both in percentage as follow up duration (3 and 4 years). The adherence rate of previous studies is summarized in **table III** and ranges between 23-88%. In most studies, the adherence rate is < 70%, lower than the rate shown in our work.

The heterogeneity of the results found in literature can be explained by the differences existing between the studies methodologies, populations, countries, allergen composition vaccines, treatment schedules, immunotherapy cost and funding. The concept of adherence is also variable; in some studies it is defined as missed doses of SCIT, while in others as stopping SCIT without medical approval.

When compared with previous published data, and according with the definition of good adherence, our adherence rate can be considered as acceptable. These good results can be explained by the existence of an Immunotherapy Center in our Outpatient Clinic where we try to promote a close and genuine patient-physician relation. There is always a support physician for SCIT administration that facilitates physician-patient communication, helping with any problem or doubt, namely addressing questions about the treatment itself, adverse reactions or any other patient's doubt. We provide a weekly schedule with extended hours in order to offer several options to SCIT administration and try not to interfere with regular working hours. We also have a direct phone number that patients can call and contact us easily. Frequent visits at our Center permit that our professionals (nurses and physicians) can enhance adherence during the visits, offering a continuous education on SCIT principles.

No statistical differences were found in our study between adherent and non-adherent groups in what concerns age, gender, clinical diagnosis or allergenic composition of the SCIT. Although it was not significant, we observed a decrease of SCIT

Table III *- Adherence to treatment in subcutaneous immunotherapy studies.*

Study	**sample (n)**	**age group**	**study duration (follow-up)**	**adherence rate (%)**
Cohn et al., 1993 (9)	217	adults	4 years	50
Lower et al., 1993 (10)	315	children	4 years	56
Donahue et al., 1999(11)	603	children and adults	4 years	33
Rhodes, 1999 (12)	1033	adults	3 years	88
More et al., 2002 (13)	381	children and adults	3 years	77
Pajno et al., 2005 (14)	1886	children	3 years	89
Hankin et al., 2008 (15)	520	children	3 years	47 (1st year) 16 (3rd year)
Hsu et al., 2012 (16)	139	adults	4 years	55
Guedechea-Sola et al., 2013 (17)	156	adults	5 years	63
Kiel et al., 2013 (18)	2796	adults	3 years	23
Silva et al., 2014 (19)	122	children and adults	4 years	54
Gelincik et al., 2017 (20)	204	adults	3 years	73
Lemberg et al., 2017 (21)	207	children and adults	3 years	68
Yang et al., 2018 (22)	311	children and adults	3 years	64.6
Tat, 2018 (23)	95	adults	3 years	65
Lee et al., 2019 (24)	1162	children and adults	3 years	80

suspension in younger patients. In literature, results regarding demographic and clinical data are also very variable. Tat also has not found differences in age or gender between the two groups (23). Rhodes found a significant correlation between age and gender: non-adherent patients were younger than adherent and males were more frequently non-adherents than females (12). More et al. confirmed Rhodes findings in what concerns age (13). On the other hand, Yang et al. concluded that children had higher adherence to SCIT than adults and did not found any other correlation with gender (22). Gelincik et al. concluded that adherence was higher in female patients. Age, clinical diagnosis and the type of allergen extract used for SCIT did not influence the adherence rate (20). Donahue et al. reported a higher adherence in patients with both asthma and rhinitis then in those with either of them (11). However, More et al. and Yang et al. have not found a correlation between adherence and kind of respiratory disease (13, 22).

Lemberg et al. concluded that patients who adhere to immunotherapy in the first year of the treatment are more likely to complete it (21). Their conclusion is in agreement with our data, where more than half of the non-adherent patients discontinued the treatment during the first year.

In order to improve adherence to treatment, it becomes particularly important to identify patients who are likely to be non-adherent and find out the reasons for stopping the treatment. The reasons for SCIT suspension are also variable among the literature; there is a lot of heterogeneity and the identified factors vary depending on the countries and populations involved.

In our study, we evaluated not only the main reasons for SCIT suspension, but we also evaluated it in separate years since the beginning of SCIT. Economic reasons were the main cause of drop-outs, responsible for 47.9% of immunotherapy suspension in a global way and for 60% of SCIT suspension in the first year. These results are in agreement with another study conducted in the north of Portugal in 2014, which reported the treatment cost as the main reason for abandoning SCIT in 59% of participants (19). To our knowledge, these are the only studies evaluating the reasons for SCIT non-adherence in Portugal, where SCIT cost ranges from 250 - 350 €/year, without reimbursement in most cases. This amount does not include the expenses related to the administration of the treatment and transportation to the hospital. Similarly, an Italian study conducted in 2005 also concluded that the cost of SCIT was the most common cause of treatment withdrawal, responsible for 39.6% of SCIT drop-outs (14). In 2011, Vaswani et al. reported a rate of 40% of suspension due to SCIT costs, especially inadequate or nonexistent insurance coverage (25). In his study, Tat also concluded that a main reason for SCIT suspension was the delayed reimbursement by health insurance (23).

Another important adherence factor is the patient's perception of clinical improvement. It is associated with his/her knowledge of treatment and the expectation of the time from initiation to symptom relief and the degree of improvement to be achieved. In our study, individual perception of absence of clinical improvement was the second leading cause of treatment withdrawal, resulting in approximately 23% of treatment discontinuation and being the main reason of suspension during the second and third year of SCIT. Gelincik et al. in their study referred the lack of efficacy as a major cause of SCIT cessation with a percentage of 66.7% (20). Silva et al. found a percentage of almost 27% due to lack of efficacy (19). Yang et al. described a discontinuation rate of 25.5% secondary to treatment inefficacy (second more frequent cause in their study) (19). Tat described a withdrawal of 14.8% of patients secondary to lack of efficacy (23).

In our study, 11.6% of patients preferred a change to sublingual immunotherapy due to SCIT inconvenience, namely, route of administration and need of monthly hospital visits. Although it was not the main reason for suspension of SCIT in the present study, treatment inconvenience is described in many studies as the main reason for treatment abandonment (9,12,16) with proportions ranging from 35 to 65%. Tat described a percentage similar to ours: 14.8% (23).

It is crucial to ensure a high adherence rate to SCIT, prior to its prescription, to inform patients about goals, risks, duration of treatment, direct and indirect costs and potential inconvenience related with the treatment (travel to appointments, skipping work). These aspects are crucial for patient's involvement in the decision to initiate SCIT. Frequently, the patient's expectations do not coincide with those of the physician. Sade et al. concluded that 39% of patients under SCIT expected full recovery, 35% expected some improvement, 16% expected prevention of the development of new allergies and 10% expected protection against the onset of asthma. In what concerns the patient's knowledge about the duration of treatment, 60% were unaware of the optimal duration and only 10% were expecting several years of therapy. These data indicate that patients were not informed about the principles of treatment with SCIT. Another conclusion was that patients who initiated treatment within the previous 6 months were more informed about it that patients receiving therapy for a longer period of time (26), reinforcing the necessity to evaluate these patients periodically.

To our knowledge, this is the second study made in Portugal on SCIT adherence, namely determination of the adherence rate and the reasons responsible for its suspension. Our study has a large sample with 323 patients. Concerning suspension factors, we evaluated these factors in a global manner and also performed an individualized analysis per year, aiming to identify and group the main causes of SCIT withdrawal and trying to be more attentive in these aspects, preventing SCIT suspension.

Limitations

The study design may limit the results: it is a retrospective study performed in one center; there was an exclusion of al-

most half of the total population due to lack of clinical information about SCIT administration. Moreover, our definition of adherence may differ from those of other studies, which can lead to some difficulty in comparing factors associated with immunotherapy adherence between the reported results. Also, this study does not consider failures / inadequate doses of allergen in SCIT administration as non-compliance of treatment.

More evidence is needed from larger samples in prospective studies, where we can get more detailed information addressing all dimensions of adherence. In addition, the definition of adherence and non-adherence to immunotherapy should be addressed in future immunotherapy guidelines.

Conclusions

The adherence rate in our study can be considered high when compared with other real-world rates, while economic reasons, followed by lack of efficacy and SCIT inconvenience were the main causes for patient's non-adherence. Informing the patients about the progress of the allergic disease and immunotherapy program may help to improve compliance. Well-informed patients are less likely to drop SCIT, once they can follow a long-lasting treatment which takes to a gradual symptom improvement.

Conflict of interests

The authors declare that they have no conflict of interest.

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Two case reports of delayed-allergic reactions to clindamycin confirmed with a positive lymphocyte transformation test

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Summary
Clindamycin is widely used in the prophylaxis and treatment of infections due to its broad spectrum of antimicrobial activity. Hypersensitivity to clindamycin seems to be not very common (less than 1% of drug-allergic reactions) and it mostly appears as delayed T-cell mediated. For the diagnosis, skin testing is considered to be highly sensitive and rather safe, but cutaneous and systemic reactions have been described. Provocation test is considered the "gold standard". However, it includes the possibility of severe reactions. We reported two cases of delayed allergic reaction to clindamycin confirmed with a positive lymphocyte transformation test, showing this in vitro test like a promising diagnostic method because of its usefulness and safety.

Introduction

Clindamycin is a lincosamide antibiotic that binds exclusively to the 50s subunit of bacterial ribosomes and suppresses intracellular protein synthesis. It is widely used in the prophylaxis and treatment of infections due to its broad spectrum of antimicrobial activity. Hypersensitivity to clindamycin seems to be not very common (less than 1% of drug-allergic reactions) (1), with mostly non-immediate or delayed ones: drug rash with eosinophilia and systemic symptoms (DRESS) (2), symmetric drug-related intertriginous and flexural exanthema (SDRIFE) (3), drug-induced hypersensitivity syndrome (DIHS) (4), generalized maculopapular exanthema (5), anaphylaxis (6) and acute generalized (7) and localised exanthematous pustulosis (8) have been described.

The diagnostic approach includes a detailed medical history, clinical examination, and skin testing and/or oral challenge with clindamycin. Lymphocyte transformation test (LTT) in general has been shown to be more sensitive than skin testing for non-immediate reactions diagnosis (9,10,11), although there are only few studies that analyze the LTT in allergy to betalactams or quinolones, so its diagnostic value for other antibiotics remains uncertain (12).

We present two different cases of delayed allergic reaction to clindamycin with maculopapular exanthema in which LTT confirmed clindamycin as the culprit agent.

Clinical cases

Case 1. A 64-year-old woman who came to the allergy department from the emergency department to be studied for a possible allergy to clindamycin. She denied any past history of urticarial episodes or adverse reactions to the ingestion of food or medication. In September 2013 she took clindamycin for a

dental infection. After the fifth dose she developed a cutaneous eruption that began in the thighs, with erythematous pruritic plaques that spread through her back and trunk day by day in spite of the clindamycin discontinuation. She was treated with high doses of prednisone for several weeks. She had no fever or systemic symptoms. Laboratory studies did not find leukocytosis, eosinophilia, kidneys failure or elevated liver enzymes. Skin prick test (150 mg/ml) and intradermal test (1.5 mg/ml and 15 mg/ml) (13,14,15) with clindamycin results were negative after 30 minutes, 24 and 48 hours. Patch testing of skin with 10% clindamycin in petrolatum at 48 and 96 h according to the Spanish Society of Allergy and Clinic Immunology criterion, was also negative (13). As the patient refused to undergo any other in vivo tests, an oral challenge with the culprit drug was not performed.

Case 2. A 56-year-old man, who came to the allergy department from his general practitioner to study a possible allergy to clindamycin. He had not allergic background. His medical history was significant for hypertension, type 2 diabetes and hyperlipidemia. His long-term drug therapy consisted of metformin, acetylsalicylic acid, olmesartan / amlodipine and simvastatin. In January 2018 he had dermatitis in his legs by the application of hydrocortisone with broponol. He received clindamycin as treatment and three days after the cutaneous eruption spread through his body, except the head, with desquamation in his lower limbs. He was studied in dermatology being diagnosed with toxicodermia. He improved with systemic prednisone but he went worse after prednisone discontinuation. Laboratory studies found leukocytosis and eosinophilia (2100/µL) but no kidney failure or elevated liver enzymes. Cutaneous biopsy was not performed. In March 2018 he arrived to allergy department being asymptomatic. Patch testing of skin with 1% clindamycin in petrolatum was negative at 48 and 96 hours.

Material and methods

In an attempt to clarify the underlying mechanism, 3 months after the reaction we performed the LTT with clindamycin in both patients. The LTT using 6 different concentrations of clindamycin (0.01 - 250 µg/ml) was performed.

Briefly, proliferation of lymphocytes from the allergic patients was measured as previously described (16,17,18). Mononuclear cells were separated over a density gradient (Histopaque 1077, Sigma-Aldrich) from fresh peripheral blood and were incubated for 6 days at 10^6 cells/mL in triplicate with 6 different concentrations of clindamycin. Phytohemagglutinin (5 µg/mL) was used as a positive control. For the final 18 hours of the incubation period, proliferation was determined by the addition of (^{3}H) thymidine (0.5 µCi/well). Stimulation index (SI), defined as the ratio between the mean values of counts per minute in cultures with antigen and those obtained without antigen, calculate the proliferative responses. The positive response is defined as an SI ≥ 2.

Results

In both patients, the result of the LTT was positive, with a SI of 5.9 at a concentration of 0.01 µg/ml and with SI of 13.1 at a concentration of 250 µg/ml, respectively (**table I**). LTT with clindamycin in four controls showed no proliferative responses. From this finding, we diagnosed maculopapular rash as delayed hypersensitivity to clindamycin.

Discussion

In drug hypersensitivity, the diagnostic approach usually includes a detailed clinical history, which is not always possible and can be unreliable. This is usually followed by appropriate in vivo tests (skin and/or drug provocation test). Although skin testing with this drug is considered to be highly sensitive and rather safe, cutaneous and systemic reactions have been described (19). Moreover, patch testing sensitivity in contact allergy is between 60-80%. They are also helpful for the study of some non-immediate adverse drug reactions, although they suffer from a lack of standardization. Sensitivity in non-betalactam antibiotics is low and there is also a high rate of false positive results due to irritation (9). Provocation test is considered the "gold standard" to establish or exclude the diagnosis of allergy to a certain substance, however, it includes the possibility of severe reactions.

Table I *- Stimulation index with different concentrations of clindamycin, in our two patients (1 and 2), and in four non-allergic to clindamycin controls.*

	Stimulation index					
clindamycin	0.01 µg/ml	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml
patient 1	5.9	2.4	1.8	1.2	2.3	-
patient 2	-	-	2.8	1.1	6.4	13.1
controls (n = 4) mean ± SD	-	-	0.8 ± 0.2	0.9 ± 0.5	0.8 ± 0.3	0.8 ± 0.2

SD, standard deviation; µg/ml, micrograms/milliliter.

Given the limitations of in vivo tests, they can be helpful for diagnosis, and are the only alternative method when in vivo tests are not recommended. They are essential to clarify drug allergy status, despite having suboptimal sensitivity. The most widely employed technique for diagnosing non-immediate reactions is LTT. Its main disadvantage is that an in vitro proliferation of T cells to a drug is difficult to transfer to the clinical situation and that the test *per se* is rather cumbersome and technically demanding. In addition, its sensitivity is limited (for β-lactam allergy it is in the range of 60-70%), although it is higher than that of other test for drug hypersensitivity diagnosis (9). LTT in general has been shown to be more sensitive than skin testing for non-immediate reactions diagnosis (9,12).

In 2012, Nakamura et al (4) reported a case of delayed DIHS/DRESS due to clindamycin intake with a positive LTT (stimulation index of 17.5 the tenth day after the DRESS start) but also with a positive skin patch test.

To our knowledge, these are the first cases reported of maculopapular rash induced by clindamycin with a positive LTT and negative skin tests and since then, no other positive results have been published. However, further studies are needed to assess the validity of the LTT in allergic reactions to clindamycin.

Conflict of interests

The authors declare that they have no conflict of interest.

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