EDRA | Issn 1764-1489 | Volume 58 N. 6/2026 – November 2026

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAIITO | ASSOCIAZIONE ALLERGOLOGI IMMUNOLOGI ITALIANI TERRITORIALI E OSPEDALIERI
THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA



6/2026

2025 Journal Impact Factor: 2.5

Sensitization to aeroallergens and asthma severity in the tropics

Basophil activation test in the diagnosis of drug-induced hypersensitivity reactions: a retrospective study of Slovak patients

Allergy evaluation in hypersensitivity to platinum compounds and taxanes: a 5-year experience

Understanding beliefs about inhaled medication in patients with persistent asthma: a cross-sectional analysis of the INSPIRERS studies

Transitioning from nonspecific therapy to berotralstat in hereditary angioedema type I: real world data from three patients within the same family

Severe chronic spontaneous urticaria with low total IgE levels: is the response to omalizumab always delayed or absent?

Redefining reference intervals for baseline serum tryptase: found the solution?

www.eurannallergyimm.com

European Annals of Allergy and Clinical Immunology

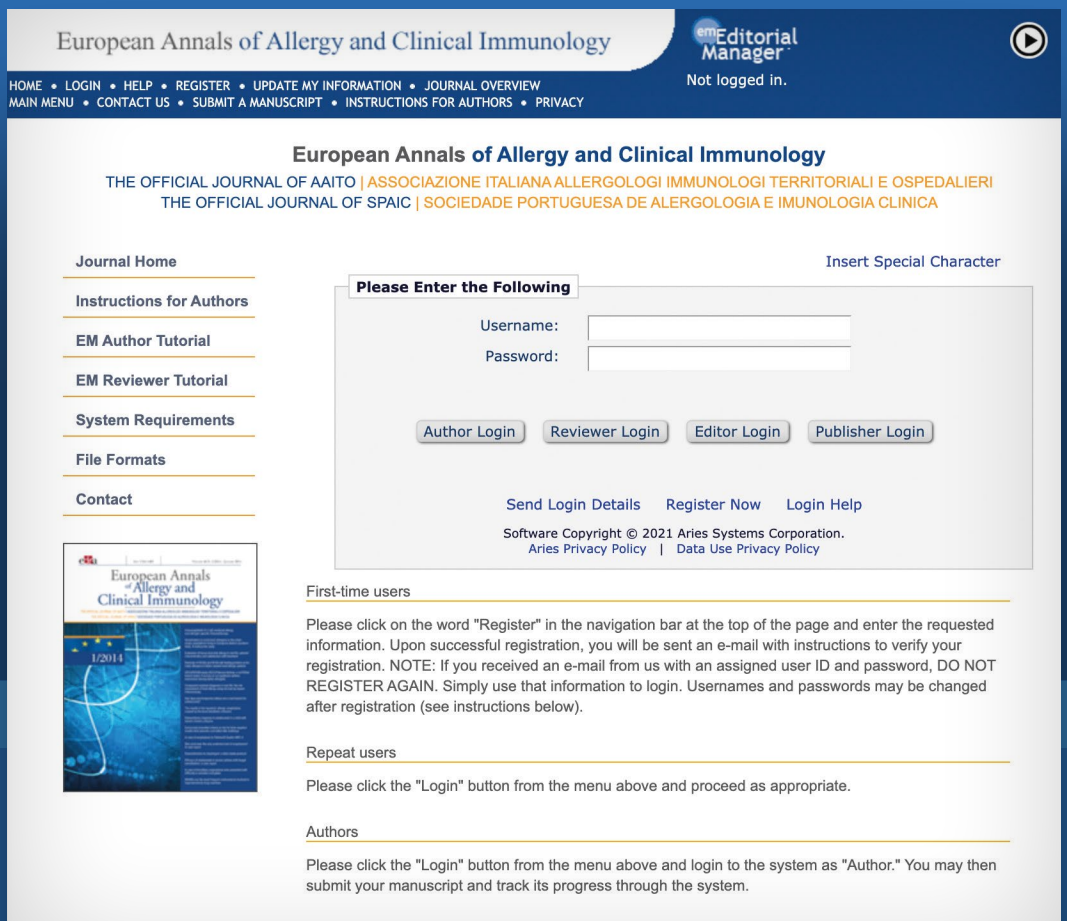
The online submission system

European Annals of Allergy and Clinical Immunology uses an online submission and review system for all papers evaluation.
Electronic submission allows a more efficient processing of manuscripts and offers Authors the option to track the progress of the review process whenever they need to. The link to the editorial system is http://eaaci.edmgr.com, it is also available on the Journal website: ***www.eurannallergyimm.com***.

The Authors are invited to submit their manuscripts through the online editorial system; manuscripts sent by e-mail, post or fax are not considered for publication.
All the Authors should read carefully the Guide for Authors before starting their submissions. Full information about the manuscript preparation are available on the Journal website. During submission, Authors will be first asked to select the article type, enter the manuscript title and provide Author information. Through a menu, a general topic area should be selected: these will help to match manuscripts to the best available editors and reviewers.Reviewers will access papers via the editorial system platform and will be invited and sent to it by email.

Full Authors Guidelines and the online Submission System link, are available on the Journal website:

www.eurannallergyimm.com



European Annals of Allergy and Clinical Immunology

www.eurannallergyimm.com

THE OFFICIAL JOURNAL OF AAIITO
ASSOCIAZIONE ALLERGOLOGI IMMUNOLOGI ITALIANI TERRITORIALI E OSPEDALIERI

THE OFFICIAL JOURNAL OF SPAIC
SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA

EDITORS IN CHIEF

M. B. Bilò (Italy)
F. S. Regateiro (Portugal)

DEPUTY EDITORS

R. Rodrigues Alves (Portugal)
D. Villalta (Italy)

ASSOCIATE EDITORS

R. Asero (Italy)
M. Branco Ferreira (Portugal)
P. Carreiro-Martins (Portugal)
L. Cecchi (Italy)
E. Scala (Italy)
D. Solé (Brasil)
G. Sturm (Austria)

EDITORIAL BOARD

I. Agache (Romania)
I. Annesi Maesano (France)
L. Antonicelli (Italy)
G. Azizi (Iran)
L.M. Borrego (Portugal)
K. Brockow (Germany)
S. Bavbek (Turkey)
E. Cichocka-Jarosz (Poland)
M. Cugno (Italy)
L. Delgado (Portugal)
P. Demoly (France)
G. D'Amato (Italy)
S. Durham (UK)
M. Faber (Belgium)
M. Fernandez-Rivas (Spain)
J. Fonseca (Portugal)
ZS. Gao (China)
G.P. Girolomoni (Italy)
E. Goudouris (Brasil)
A. Grumach (Brasil)
G. Kostantinou (Greece)
F. Levi-Shaffer (Israel)
M. Maurer (Germany)
L. Mayorga (Spain)
C. Micheletto (Italy)
M. Morais de Almeida (Portugal)
G. Moscato (Italy)
A. Musarra (Italy)
C. Nunes (Portugal)
M. Ollert (Lussemburgo)
P. Parronchi (Italy)
G. Passalacqua (Italy)
E. Pedro (Portugal)
A. Perino (Italy)
O. Quercia (Italy)
A. Romano (Italy)
G. Scadding (UK)
A. Todo Bom (Portugal)
A. Tedeschi (Italy)
R. van Ree (Netherland)
D. Villalta (Italy)
S. Voltolini (Italy)

FOUNDERS

F. Bonifazi (Italy)
A. Sabbah (France)

EDRA
CONTEMPORARY HEALTH

Editorial Coordinator

Marco Malagutti

Publishing Editor

Jessica Guenzi
editorialoffice@eur-ann-allergy-clin-immunol.com
Ph. 0039 3491716011

Sales

dircom@lswr.it

Edra Media S.r.l.

Viale Forlanini, 21
20134 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edramedia.it

"European Annals of Allergy and Clinical Immunology" registered at Tribunale di Milano - n. 336 on 22.10.2014

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved.

To read our Privacy Policy please visit www.edramedia.it/privacy-policy.html

We are Crossref Sponsored Member

The contents of this Journal are indexed in PubMed, Scopus, Embase and Web of Science®

AAIITO
Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri

Directory Board

President
Francesco Murzilli

Designated President
Danilo Raffaele Villalta

Vice President
Donatella Bignardi

Treasurer
Oliviero Quercia

Past President
Lorenzo Cecchi

Members
Ignazio Brusca
Marcello Cilia
Maurizio Franchini
Francesco Madonna
Alessandro Maria Marra
Giuseppe Pingitore
Giuliana Zisa

SPAIC
Sociedade Portuguesa de Alergologia e Imunologia Clínica

Directory Board

President
Pedro Carreiro Martins

Past President
Ana Morête

Vice Presidents
João Gaspar Marques
Frederico Regateiro
Diana Silva

Treasurer
Rodrigo Rodrigues Alves

Secretary-General
José Alberto Ferreira

Secretary-Adjunct
Joana Cosme

Table of Contents

Original articles

Sensitization to aeroallergens and asthma severity in the tropics 243
Layssa Fabiana Fernandes de Matos, Gabriela Pimentel Pinheiro das Chagas, Cinthia Vila Nova Santana, Aline Carolina Rodrigues Santos, Luiz Henrique Azevedo Silva, Miquéias Saimon, Giovana Coletto Segger, Álvaro A. Cruz, Jamille Souza Fernandes Chiarelotto

Basophil activation test in the diagnosis of drug-induced hypersensitivity reactions: a retrospective study of Slovak patients 252
Mira Horváthová, Jana Surmová, Nikola Madrová, Michaela Szabová

Allergy evaluation in hypersensitivity to platinum compounds and taxanes: a 5-year experience 263
David Longhino, Caterina Sarnari, Gloria Andriollo, Arianna Aruanno, Angela Rizzi, Eleonora Nucera, Alessandro Buonomo

Understanding beliefs about inhaled medication in patients with persistent asthma: a cross-sectional analysis of the INSPIRERS studies. 269
Catarina Novais, Ana Margarida Cruz, Ana Margarida Pereira, Rute Almeida, Rita Amaral, João Almeida Fonseca, Cristina Jácome

Letters to the Editor

Transitioning from nonspecific therapy to berotralstat in hereditary angioedema type I: real world data from three patients within the same family. 279
Denis Vincent, Arije Ghanam

Severe chronic spontaneous urticaria with low total IgE levels: is the response to omalizumab always delayed or absent? 283
Riccardo Asero

Redefining reference intervals for baseline serum tryptase: found the solution?. 286
Danilo Villalta, Mariaelisabetta Conte, Daniela Visentini, Matteo Furlani, Francesco Ellero, Roberta Metani, Fiorenza Pesente, Paolo Metus

EDRA ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.6, 243-251, 2026

Layssa Fabiana Fernandes de Matos[1], Gabriela Pimentel Pinheiro das Chagas[2], Cinthia Vila Nova Santana[2,3], Aline Carolina Rodrigues Santos[1], Luiz Henrique Azevedo Silva[1], Miquéias Saimon[1], Giovana Coletto Segger[1], Álvaro A. Cruz[2], Jamille Souza Fernandes Chiarelotto[1,2]

Sensitization to aeroallergens and asthma severity in the tropics

[1]Center for Biological and Health Sciences, Federal University of Western Bahia, Barreiras, Bahia, Brazil
[2]ProAR Foundation and Federal University of Bahia, Salvador, Bahia, Brazil
[3]Bahiana School of Medicine and Public Health, Salvador, Bahia, Brazil

Key words

Allergic asthma; eosinophilia; IgE antibodies specific; polysensitization; severe asthma.

Corresponding author
Jamille Souza Fernandes Chiarelotto
Center for Biological and Health Sciences
Federal University of Western Bahia
Rua da Prainha 1326
Morada Nobre, Barreiras, Bahia, Brazil 47810-047
ORCID: 0000-0003-1169-0724
E-mail: sf.jamille@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.433

Impact statement

Asthma is a disease with diverse phenotypes. The association between sensitization to perennial aeroallergens and most specifically A. fumigatus with the severity of asthma indicates they may be relevant risk factors for severe asthma and should inform personalized clinical management.

Summary

Background. *The allergic asthma phenotype is the most extensively investigated. However, few studies have explored the association between sensitization to different aeroallergens, asthma severity and blood eosinophil counts, an important biomarker of Type 2 inflammation. This study aims to evaluate associations between sensitization to aeroallergens, severity of asthma, eosinophilic phenotypes and age of onset of symptoms.* ***Methods.*** *This was a cross-sectional study involving a tropical urban population sample. We included 450 individuals with mild asthma (MA) and 441 with moderate to severe asthma (MSA). Serum-specific IgE to nine aeroallergens was measured by the ImmunoCap™. We considered a cut-off point of 0.70 KU/L for mites and 0.35 KU/L for all other allergens. Blood eosinophilia was considered if the count was > 260 cells/mm^3, and the age of onset was defined as early (EO < 12 years) and late (LO ≥ 12 years).* ***Results.*** *Patients with asthma sensitive to dust mites, B. germanica and those with polysensitization are 1.6 to 1.8-fold more likely to have MSA, while in cases of sensitization to A. fumigatus this probability increases to 3-fold. Regarding the eosinophilic phenotype, individuals with MA sensitized to dust mites, B. germanica, dog epithelium and polysensitized individuals are more likely to present the eosinophilic phenotype ($p < 0.05$). However, we did not observe any association between sensitization to different aeroallergens and age of symptom onset ($p > 0.05$).* ***Conclusions.*** *We conclude that polysensitization or sensitization to mites, B. germanica, and mainly to A. fumigatus, are associated with the severity of asthma. Only in individuals with MA, sensitization to dust mites, B. germanica, dog epithelium and polysensitization is associated with eosinophilic asthma. No sensitization profile was associated with the age of onset of asthma symptoms.*

Introduction

Asthma is a disease that has a chronic inflammatory nature, in general, being characterized by narrowing of the airways, thickening of the basement membrane, increased production and secretion of mucus, and bronchial hyperresponsiveness, which can reduce lung function over the years (1, 2). Commonly, the patient with asthma presents a clinical history of respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough that vary in intensity and over time. Asthma severity can range from a mild condition, with almost imperceptible symptoms, to a severe condition, with persistent and troublesome symptoms that significantly impair the patient's quality of life.

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

Despite the diagnosis being mostly clinical and supported by lung function measurements, previous studies have reported the complexity and heterogeneity of asthma as a disease comprising several phenotypes (3). Asthma phenotypes often overlap and include atopic asthma, non-atopic asthma, late-onset asthma, fixed airflow-limited asthma, asthma with obesity, eosinophilic asthma, neutrophilic asthma (1, 3). Because of these phenotypic characteristics, it was noted that asthma management should not be tailored only to the severity of the disease but individualized and modified according to the patients' response to treatment and guided by the inflammatory phenotype alongside with several clinical and behavioral traits (4, 5).

The allergic asthma phenotype is the most frequent among the phenotypes observed in asthma (6, 7). Its response is predominantly Type 2, with production of IL-4, IL-5, and IL-13 cytokines and specific IgE antibodies. Such molecules will contribute to the pathogenesis of the disease with the production of mucus, inflammation, bronchoconstriction, and bronchial hyperreactivity, as reviewed in Lambrecht and Hammad, 2021 (8).

Approximately 65 to 80% of the patients diagnosed with asthma have some type of sensitization to aeroallergens, with sensitization to indoor aeroallergens being more frequent, such as dust mites, dog and cat epithelium, insects (cockroaches), fungi, and some rodents (9-11). Furthermore, this sensitization to indoor airborne allergens have been associated with greater severity and morbidity of asthma in areas of temperate climate (12, 13).

Thus, our objective was to evaluate the association between sensitization to different aeroallergens with the severity of asthma in a tropical environment, to understand which sensitization profile might correlate with the severity of the disease, as well as to investigate possible associations between the sensitization profile, blood eosinophilia and age of onset of symptoms.

Materials and methods

Study population

This was a cross-sectional study involving a tropical urban population conducted between January 2013 and July 2015 at the ProAR Central Clinic in Salvador, Bahia, Brazil. We included individuals with mild asthma (MA) and moderate to severe asthma (MSA), of both sexes, aged between 18 and 60 years. Individuals with MSA were part of the Program for Asthma Control in Bahia (ProAR), a referral clinic for severe asthma in Salvador, Bahia, Brazil. We did not include pregnant women, patients with poor understanding and verbal communication, patients with incomplete or inconsistent information or data. Furthermore, patients with a history of stroke, congestive heart failure, myopathy, pulmonary disease (besides asthma), or other diseases that could interfere with the assessment of asthma severity and control were excluded, as well as those who were over 60 years old. Since aging is associated with physiological changes in lung function and a higher prevalence of the aforementioned comorbidities, immunosenescence might also bias the results of specific IgE measurements (14, 15). Patients with MSA were classified according to the presence of any of the following criteria at their enrollment in ProAR: 1) report of daily symptoms, 2) frequent exacerbations or nighttime symptoms, 3) limitation in their physical activities, 4) reduced lung function (FEV1 or Peak Expiratory Flow ≤ 60%) or variability in FEV1 or Peak Expiratory Flow > 30%. At the time of blood collection, at least 6 months after enrollment, all individuals with MSA were under treatment and were using a combination of medium or high-dose inhaled corticosteroids (800 mcg to 1,600 mcg of Budesonide or equivalent) and a long-acting beta-2 agonist (12 or 24 mcg of formoterol or 100 mcg of salmeterol daily). These individuals with MSA were classified according to the criteria established by GINA 2012 (16). Individuals with MA were invited to volunteer through advertisements at health care centers and public transport and had their diagnosis validated by an expert in the field, according to the criteria of GINA 2012 (16). They were not under regular treatment with inhaled corticosteroids. In addition, the asthma diagnosis of all patients with MSA was validated by two specialists, and the opinion of a third specialist was also requested in case of disagreement regarding the diagnosis. A blood sample was also collected from the patients to perform the complete blood count and measure specific IgE (sIgE).

The study was approved by the Ethics Committee of Maternidade Climério de Oliveira, Federal University of Bahia, Salvador, Bahia, Brazil (License Number: CONEP nº15782, CEP 095/2009 and 095/2012). All individuals who were recruited and agreed to participate in the study signed an informed consent form.

Confounding variables

Potential confounding variables considered in this study included age, sex, exposure to wood stove smoke, diagnosis of rhinitis, gastroesophageal reflux disease (GERD), and obesity. The diagnosis of rhinitis and GERD were obtained during a consultation with a specialist, considering reports of symptoms and previous medication use. Obesity was defined by a body mass index (BMI) ≥ 30 kg/m^2 and abdominal circumference measurement (≥ 88 cm for women and ≥ 102 cm for men) (17, 18). These variables were collected to account for potential confounding effects in the association analyses between asthma and aeroallergen sensitization. Age and sex are fundamental demographic variables that may influence a wide range of clinical outcomes, including asthma severity and specific IgE levels (11, 14, 15). Environmental exposures, such as wood-stove smoke, have been associated with airway inflammation and inadequate asthma control (19). Comorbidities, including rhinitis and GERD, are common in asthma patients and may contribute to increased disease severity, while obesity is consistently associated with poorer asthma control (11, 20). Given these established associations, these variables were included in the model to minimize confounding effects. It is noteworthy that we did not

include tobacco smoke exposure due to the low frequency of active and passive smoking in our study population (11, 21).

Measurement of specific IgE (sIgE), definition of eosinophilia and age at onset of asthma symptoms

Sensitization to aeroallergens was determined by the presence of sIgE in serum by the fluorescence solid phase immunoassay (ImmunoCAP™) technique (Thermo Fisher Scientific, Uppsala, Sweden). The main aeroallergens evaluated were *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus fumigatus*, *Penicillium notatum*, *Periplaneta americana, Blatella germanica,* dog epithelium, and rat epithelium and urine. Cat epithelium was not included in this study because it has similar clinical relevance to dog epithelium among adult individuals with asthma in Brazil (22), in addition to project cost constraints. We considered a cut-off point of 0.70 KU/L for mites and 0.35 KU/L for all other allergens, as recommended by the manufacturer. The cut-off of ≥ 0.70 kU/L for mites sIgE was chosen because in tropical regions such as Brazil, low levels of dust mite-specific IgE may reflect cross-reactivity with helminth antigens rather than true allergic sensitization, thus reducing specificity in the definition of atopy (23-27). Furthermore, Hong *et al.* (2018) demonstrated, using ROC curve analysis, that the optimal point for *Dermatophagoides pteronyssinus* is approximately 0.69 kU/L (28), very close to our chosen cut-off.

In the present study, monosensitization was defined as sensitization to a single aeroallergen, whereas polysensitization was defined as sensitization to two or more aeroallergens, regardless of whether they were from different categories (*e.g.*, mites and cockroach) or from the same type (*e.g.*, different mite species). The criterion we established for eosinophilic asthma was a peripheral blood eosinophil count greater than 260 eosinophils/mm^3 (29). Regarding the age of onset of asthma symptoms, the 12-year limit was used to define the two phenotypes: early age of onset (EO < 12 years) and late age of onset (LO ≥ 12 years) (30, 31).

Statistical analysis

Statistical analyses were performed using SPSS 26.0. After performing the Kolmogorov-Sminorv normality test, we noticed our data had a non-parametric distribution. Thus, we used the Mann-Whitney test to compare two groups. For analysis of categorical data, we performed Fisher's exact test. Regarding logistic regression, we adjusted for confounding variables such as age, sex, exposure to wood stove smoke, diagnosis of rhinitis, GERD, and obesity. Moreover, each logistic regression model was built including one sensitization variable and adjusted for confounders. No simultaneous comparison of all sensitization categories was performed. The continuous variables were expressed in median (1st quartile – 3rd quartile). All statistical tests were two-tailed and a $p < 0.05$ was considered statistically significant.

Results

Characteristics of the study population

We evaluated 891 individuals, 450 with MA and 441 with MSA, of both sexes, aged between 18 and 60 years. **Table I** shows the characteristics of the study population. We observed that the median age of individuals with MSA was higher when compared to individuals with MA. Most participants were female,

Table I *- Sociodemographic and clinical characteristics of the study sample.*

	Mild Asthma (MA) n = 450	Moderate to Severe Asthma (MSA) n = 441	P-value
Age*[a]	35 (26.0-47.0)	52 (43.0-61.0)	**< 0.0001**
Female sex n (%)**	349 (77.5)	354 (80.3)	0.32
Age of onset of asthma*[a]	7.0 (1.0-19.0)	10.0 (2.0-26.0)	**< 0.01**
Obesity n (%)**[b]	106 (23.6)	176 (39.9)	**< 0.0001**
Rhinitis n (%)**	406 (90.2)	416 (94.3)	**< 0.05**
GERD n (%)**	288 (64)	311 (70.5)	**< 0.05**
FEV_1 Pre-BD (%)*[a]	82.2 (72.0-90.8)	61.0 (49.1-71.9)	**< 0.0001**
Total IgE (UI/mL)*[a]	212.0 (86.2-529.4)	449.3 (144.3-499.3)	**< 0.01**
Eosinophils/mm^3*[a]	235.0 (131.0-394.0)	248.5 (143.0-392.3)	0.21
Eosinophils > 260 cells/mm^3 n (%)**	209 (46.4)	203 (46.0)	0.94

*Mann Whitney test; **Fisher's Exact Test; [a]median (1st quartile-3rd quartile); [b]considering body mass index (BMI) ≥ 30kg/m^2 and measurement of abdominal circumference (≥ 88 cm for women and ≥ 102 cm for men); bolded results are statistically significant at P-value < 0.05. FEV1 pre-BD, forced expiratory volume in one second pre-bronchodilator; GERD: gastroesophageal reflux disease.

with no difference between groups. Regarding the age of onset of asthma symptoms, we noticed that the median was higher in individuals with MSA than in individuals with MA. Additionally, the frequency of obesity, rhinitis, GERD and total IgE levels were higher in individuals with MSA than individuals with MA. Regarding lung function, analyzed through the assessment of pre-bronchodilator forced expiratory volume in the 1st second (Pre-BD FEV1), we observed that FEV1 in individuals with MSA was lower than in the MA group. We did not observe a significant difference in eosinophil counts nor the frequency of eosinophilic asthma in comparison to MA and MSA.

Association between sensitization aeroallergens with asthma severity and phenotypes

Table II shows the association between sensitization to relevant aeroallergens and asthma severity. Initially, we observed no signif-

Table II - *Association between sensitization to the relevant aeroallergens and asthma severity.*

	MA (n = 415)	MSA (n = 396)	P-value	OR unadjusted	OR adjusted*
B. tropicalis	239 (57.6%)	202 (51.0%)	0.06	0.76 (0.58 – 1.01)	**1.61 (1.13 – 2.29)**
D. pteronyssinus	213 (51.3%)	189 (47.7%)	0.32	0.86 (0.65 – 1.14)	**1.76 (1.23 – 2.52)**
D. farinae	227 (54.7%)	198 (50.1%)	0.20	0.83 (0.63 – 1.09)	**1.74 (1.22 – 2.47)**
A. fumigatus	13 (3.1%)	30 (7.6%)	**0.007**	**2.53 (1.30 – 4.93)**	**3.21 (1.41 – 7.26)**
P. notatum	15 (3.6%)	25 (6.3%)	0.10	1.79 (0.93 – 3.45)	1.70 (0.75 – 3.81)
Blatella germanica	113 (27.2%)	125 (31.6%)	0.19	1.23 (0.91 – 1.66)	**1.84 (1.26 – 2.67)**
Periplaneta americana	67 (16.1%)	60 (15.2%)	0.70	0.92 (0.63 – 1.35)	1.33 (0.83 – 2.12)
Dog epithelium	60 (14.5%)	65 (16.4%)	0.49	1.16 (0.79 – 1.70)	1.44 (0.91 – 2.29)
Rat epithelium and urine	4 (1.0%)	4 (1.0%)	1.00	1.04 (0.26 – 4.21)	0.58 (0.11 – 3.06)
Monosensitization	27 (6.5%)	48 (12.2%)	**0.007**	**1.97 (1.20 – 3.23)**	1.55 (0.83 – 2.84)
Polysensitization	244 (59.1%)	222 (56.2%)	0.43	0.88 (0.67 – 1.17)	**1.82 (1.27 – 2.60)**

*Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P-value < 0.05. MA: mild asthma; MSA: moderate to severe asthma; monosensitization: sensitization to a single aeroallergen; OR: odds ratio; polysensitization: sensitization to two or more aeroallergens.

Table III - *Association between sensitization to relevant aeroallergens and mild eosinophilic asthma.*

	MA Eosinophilic (n = 188)	MA Non-Eosinophilic (n = 227)	P-value	OR unadjusted	OR adjusted*
B. tropicalis	141 (75%)	98 (43.1%)	**0.000**	**3.94 (2.59 – 6.02)**	**3.39 (2.16 – 5.32)**
D. pteronyssinus	128 (68%)	85 (37.4%)	**0.000**	**3.56 (2.37 – 5.35)**	**2.87 (1.86 – 4.43)**
D. farinae	139 (73.9%)	88 (38.76%)	**0.000**	**4.48 (2.94 – 6.87)**	**3.82 (2.45 – 5.96)**
A. fumigatus	8 (4.2%)	5 (2.2%)	0.26	1.97 (0.84 – 7.47)	1.26 (0.37 – 4.26)
P. notatum	10 (5.3%)	5 (2.2%)	0.11	2.50 (0.36 – 1.90)	2.92 (0.85 – 9.96)
Blatella germanica	64 (34%)	49 (21.5%)	**0.006**	**1.87 (1.21 – 2.90)**	**1.71 (1.08 – 2.71)**
Periplaneta americana	36 (19.1%)	31 (13.6%)	0.14	1.49 (0.88 – 2.53)	1.31 (0.75 – 2.27)
Dog epithelium	40 (21.2%)	20 (8.81%)	**0.000**	**2.79 (1.57 – 4.98)**	**2.58 (1.41 – 4.72)**
Rat epithelium and urine	3 (1.59)	1 (0.44%)	0.33	3.68 (0.38 – 35.7)	4.71 (0.43 – 50.5)
Monosensitization	10 (5.3%)	17 (7.5%)	0.43	0.70 (0.31 – 1.57)	0.82 (0.35 – 1.92)
Polysensitization	147 (78.2%)	97 (42.7%)	**0.002**	**5.05 (3.25 – 7.84)**	**4.27 (2.68 – 6.80)**

**Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P-value < 0.05. MA: mild asthma; monosensitization: sensitization to a single aeroallergen; OR: odds ratio; polysensitization: sensitization to two or more aeroallergens.*

icant differences in monosensitization to mites in general (such as *B. tropicalis, D. pteronyssinus,* and *D. farinae*), cockroach (*B. germanica*), or polysensitization between individuals with MA and MSA, nor any association between these sensitizations and disease severity. However, after adjusting for confounding variables, individuals with these sensitization profiles were 1.6- to 1.8-fold more likely to have MSA. Conversely, sensitization to *A. fumigatus* was significantly more frequent in the MSA group than in the MA group. After adjustment, asthmatic individuals sensitized to this fungus had a three-fold higher likelihood of having MSA. Furthermore, we also assessed associations with eosinophilia and age at onset of symptoms. Individuals with MA sensitized to the three mites are 3-fold more likely to present the eosinophilic phenotype (**table III**). When sensitized to *Blatella germanica* and dog

Table IV *- Association between sensitization to relevant aeroallergens and moderate to severe eosinophilic asthma.*

	MSA Eosinophilic (n = 203)	MSA Non-Eosinophilic (n = 234)	P-value	OR unadjusted	OR adjusted*
B. tropicalis	99 (48.7%)	103 (44%)	**0.05**	**1.49 (1.00 – 2.22)**	1.18 (0.77 – 1.82)
D. pteronyssinus	89 (43.8%)	100 (42.7%)	0.31	1.25 (0.84 – 1.86)	0.98 (0.63 – 1.50)
D. farinae	95 (46.7%)	103 (44%)	0.12	1.37 (0.92 – 2.05)	1.05 (0.68 – 1.62)
A. fumigatus	14 (6.8%)	16 (6.8%)	0.84	1.11 (0.52 – 2.35)	1.03 (0.47 – 2.28)
P. notatum	10 (4.9%)	15 (6.4%)	0.83	0.83 (0.36 – 1.90)	0.70 (0.29 – 1.66)
Blatella germanica	59 (29%)	66 (28.2%)	0.44	1.19 (0.78 – 1.82)	0.99 (0.63 – 1.57)
Periplaneta americana	29 (14.2%)	31 (13.2%)	0.48	1.21 (0.70 – 2.11)	1.05 (0.59 – 1.89)
Dog epithelium	36 (17.7%)	29 (12.3%)	**0.05**	**1.71 (1.00 – 2.92)**	1.55 (0.88 – 2.71)
Rat epithelium and urine	2 (0.98%)	2 (0.85%)	1.00	1.26 (0.17 – 9.07)	0.72 (0.93 – 5.57)
Monosensitization	16 (7.8%)	32 (13.6%)	0.12	0.59 (0.31 – 1.13)	0.64 (0.33 – 1.25)
Polysensitization	105 (51.7%)	117 (50%)	0.15	1.35 (0.90 – 2.02)	1.06 (0.69 – 1.64)

**Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P-value < 0.05. MSA: moderate to severe asthma; monosensitization: sensitization to a single aeroallergen; OR: odds ratio; polysensitization: sensitization to two or more aeroallergens.*

Table V *- Association between sensitization to relevant aeroallergens and early-onset mild asthma.*

	MA-EO (n = 255)	MA-LO (n = 147)	P-value	OR unadjusted	OR adjusted*
B. tropicalis	156 (61.2%)	76 (51.7%)	0.07	1.47 (0.98 – 2.22)	0.98 (0.62 – 1.55)
D. pteronyssinus	143 (56.1%)	64 (43.5%)	**0.017**	**1.66 (1.10 – 2.49)**	1.17 (0.74 – 1.84)
D. farinae	149 (58.4%)	72 (48.9%)	0.07	1.46 (0.97 – 2.20)	1.01 (0.64 – 1.59)
A. fumigatus	8 (3.1%)	5 (3.4%)	1.00	0.92 (0.29 – 2.86)	0.66 (0.19 – 2.29)
P. notatum	9 (3.5%)	6 (4.0%)	0.79	0.86 (0.30 – 2.48)	0.95 (0.29 – 3.09)
Blatella germanica	70 (27.4%)	40 (27.2%)	1.00	1.01 (0.64 – 1.59)	0.85 (0.52 – 1.37)
Periplaneta americana	44 (17.2%)	23 (15.6%)	0.78	1.12 (0.65 – 1.95)	0.84 (0.47 – 1.52)
Dog epithelium	35 (15.5%)	23 (15.6%)	0.66	0.86 (0.48 – 1.52)	0.74 (0.39 – 1.36)
Rats epithelium and urine	1(0.3%)	1(0.3%)	1.00	0.58 (0.36 – 9.29)	0.69 (0.04 – 13.4)
Monosensitization	16 (6.2%)	9 (6.1%)	1.00	1.03 (0.44 – 2.40)	1.15 (0.46 – 2.85)
Polysensitization	157 (61.5%)	80 (54.4%)	0.14	1.37 (0.91 – 2.07)	0.95 (0.59 – 1.51)

**Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P-value < 0.05. EO: early onset; LO: late onset; MA: mild asthma; monosensitization: sensitization to a single aeroallergen; OR: odds ratio; polysensitization: sensitization to two or more aeroallergens.*

***Table VI** - Association between sensitization to relevant aeroallergens and early-onset moderate to severe asthma.*

	MSA-EO (n = 229)	MSA-LO (n = 210)	P-value	OR unadjusted	OR adjusted*
B. tropicalis	120 (52.4%)	81 (38.5%)	**0.002**	**1.90 (1.27 – 2.83)**	1.31 (0.84 – 2.04)
D. pteronyssinus	114 (49.7%)	75 (35.7%)	**0.002**	**1.92 (1.28 – 2.86)**	1.52 (0.97 – 2.38)
D. farinae	118 (51.5%)	79 (37.6%)	**0.002**	**1.92 (1.29 – 2.87)**	1.39 (0.89 – 2.18)
A. fumigatus	12 (5.2%)	18 (8.5%)	0.18	0.59 (0.27 – 1.26)	0.63 (0.27 – 1.50)
P. notatum	9 (3.9%)	16 (7.6%)	0.14	0.49 (0.21 – 1.15)	0.48 (0.18 – 1.25)
Blatella germanica	69 (30.1%)	56 (26.6%)	0.38	1.21 (0.79 – 1.85)	1.04 (0.64 – 1.68)
Periplaneta americana	33 (14.4%)	27 (12.8%)	0.67	1.15 (0.66 – 2.01)	1.09 (0.59 – 2.02)
Dog epithelium	35 (15.2%)	30 (14.2%)	0.78	1.09 (0.64 – 1.87)	0.90 (0.50 – 1.63)
Rat epithelium and urine	1 (0.47%)	3 (1.3%)	0.35	0.30 (0.03 – 2.96)	0.31 (0.25 – 4.00)
Monosensitization	24 (10.4%)	24 (11.4%)	0.87	0.92 (0.50 – 1.68)	1.15 (0.59 – 2.23)
Polysensitization	128 (55.8%)	93 (44.2%)	**0.006**	**1.75 (1.17 – 2.62)**	1.27 (0.81 – 1.99)

**Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P-value < 0.05. EO: early onset; LO: late onset; MSA: moderate to severe asthma; monosensitization: sensitization to a single aeroallergen; OR: odds ratio; polysensitization: sensitization to two or more aeroallergens.*

epithelium, the chances of presenting this phenotype are 1.7 and 2.5-fold greater, respectively, while the possibilities increase to 4-fold more when these individuals are polysensitized.

For individuals with MSA, we did not observe any association between sensitization to different aeroallergens and eosinophilia (**table IV**).

Regarding the age of onset of symptoms, there was no significant association with sensitization (**tables V** and **VI**). Individuals with MA sensitized to *D. pteronyssinus* and individuals with MSA sensitized to any of the three mites or polysensitized were more likely to have early-onset asthma, but after adjustments for confounding variables this association was not statistically significant (**tables V** and **VI**).

Discussion and conclusions

This study explored the relationship between sensitization to relevant aeroallergens and asthma phenotypes in a tropical city. Overall, our results indicate that sensitization to aeroallergens was significantly associated with asthma severity. Among patients with MA, sensitization was related with the eosinophilic phenotype, but not with the age of symptom onset.

Initially, we looked for associations between sensitization to aeroallergens and severity of asthma. Among the nine aeroallergens evaluated, asthmatic individuals sensitive to dust mites (*B. tropicalis*, *D. pteronyssinus*, *D. farinae*) and the *Blatella germanica* cockroach were more likely to present more severe disease. Some studies have observed that increased exposure and sensitization to aeroallergens such as the *D. pteronyssinus* mite and cockroaches in children diagnosed with asthma may be a factor that contributes to the severity and increased morbidity associated with this disease (32, 33). Li *et al*. evaluated 3,780 asthmatics between 5 and 65 years old in China, and they noted that the severity of the disease increases as the frequency of sensitization to *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* increases (34).

Our study evaluated only adults over 18 years of age, these aforementioned associations were observed only after adjusting for confounding variables. Among all tested variables, age was the only factor that modified these associations (data not shown). A plausible explanation for this finding is that specific IgE levels to aeroallergens, particularly to dust mites, tend to decrease significantly with increasing age, as previously reported by Tosca *et al*. (2017) (14). This age-related decline in IgE reactivity likely attenuated the differences in sensitization to these aeroallergens between the MA and MSA groups, which may explain the absence of significant differences in the unadjusted analyses. Thus, as described in studies with younger populations, adults sensitized to dust mites also appear more likely to develop MSA. Furthermore, it is important to highlight that we found a strong association between sensitization to *A. fumigatus* and asthma severity, confirming the findings of other studies that found that fungal sensitization is indeed associated with asthma severity, mortality, and hospital admission (35, 36).

Regarding sensitization to the epithelium of dogs and rats, we found no association with asthma severity. Our data agrees with Cacheiro-Llaguno *et al*. (2024) who observed an association between sensitization to dog epithelium and the severity of rhinitis symptoms, but not asthma (37). In addition, Schleich *et*

al. (2023) found a low proportion of sensitization to the epithelium of dogs and rats in individuals with severe asthma, similar to our study, and they did not observe an association of this sensitization with the severity of the disease (10).

The number of sensitizations is another relevant aspect to be considered, since both our study and others have observed a significant association between polysensitization and asthma severity (38, 39). This makes prescribing specific immunotherapy more challenging (37, 40), as well as taking care to reduce exposure to these aeroallergens.

Atopy is generally associated with the phenotypes of eosinophilic asthma and early-onset asthma (41). For this reason, we also investigated association between sensitization to different aeroallergens with eosinophilic phenotypes and age at onset of symptoms. We observed that individuals with MA sensitized to *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, *B. germanica,* dog epithelium and those polysensitized are more likely to present an eosinophilic phenotype than a non-eosinophilic one. However, regardless of the profile and number of sensitizations, in individuals with MSA, the chances of presenting the eosinophilic or non-eosinophilic phenotype are similar.

In relation to the age at onset of symptoms, although atopy is generally associated with onset asthma in childhood, our data showed no sensitization profile or number of sensitizations (*i.e.*, monosensitization or polysensitization) associated with early onset, regardless of asthma severity (*i.e.*, MA or MSA). This shows that, regardless of the severity of asthma, an individual presenting the first asthma symptoms in childhood or adulthood may have the same chances of being sensitized to any of the aeroallergens evaluated.

Our study has limitations. We did not perform direct comparisons between excluded and included individuals, since excluded participants had incomplete or inconsistent data or limited verbal communication, which could have affected the assessment of asthma severity and control. The cut-off count used to define eosinophilic asthma is arbitrary; however, it has been adopted in previous studies (29). Similarly, the definitions of early and late onset are also arbitrary, but they have been used by other authors (30, 31). Our analysis was not designed to directly compare all sensitization categories, thus the possibility of a Type I error cannot be excluded. Additionally, interaction terms were not explored, and model fit was not formally assessed, which we recognize as limitations to be addressed in future studies. One must also remember that being a variable disease, the classification of asthma severity is not entirely precise. Any of these limitations or a combination of them, may generate biases that may influence the results observed.

As far as we know, this is the first study to evaluate sensitization to different aeroallergens and various phenotypes in individuals with MA and MSA. Furthermore, our study is one of the few that provides this more detailed approach to atopy and asthma phenotypes in the adult population. Our findings suggest that sensitization to dust mites, *B. germanica*, *A. fumigatus* and polysensitization may be associated with asthma severity in adult asthmatic individuals. In individuals with MA, but not in MSA, sensitization to dust mites, *B. germanica*, dog epithelium and polysensitization may be associated with eosinophilic phenotype asthma. No sensitization profile was associated with the age of onset of asthma. Due to the cross-sectional nature of this study in an urban tropical population, these associations of sensitization patterns with asthma severity and phenotypes may not have external validity to different populations and environments.

Fundings

Alvaro A. Cruz was awarded a grant to constitute Center for Excellence in Asthma, Federal University of Bahia from CNPq/FAPESB (Notice 020/2009 - PRONEX – 6353 – PNX0018/2009). An additional grant was obtained by an investigator initiated proposal of Alvaro A. Cruz supported by Trust in Science, a GlaxoSmithKline's program.

Contributions

LFFM, JSFC: conceptualization, formal analysis, methodology, writing – original draft. AAC: conceptualization, funding acquisition, investigation, supervision, writing – review & editing. GPPC, CVNS: methodology, writing – review & editing. ACRS, LHAS, MS, GCS: formal analysis, methodology.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors would like to thank Juliana Viana, Aline Lima, Paula Almeida, Givaneide Lima, Valmar Bião, Ila Muniz and Ana Tereza Campos for assisting in the recruitment and evaluation of patients. Ajax Atta and Isabela Oliveira for the performance of the specific IgE by ImmunoCAP.

References

1. GINA. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. 2024. Available at: https://ginasthma.org/wp-content/uploads/2024/05/GINA-2024-Strategy-Report-24_05_22_WMS.pdf.
2. Herscher ML, Wisnivesky JP, Busse PJ, Hanania NA, Sheng T, Wolf MS, et al. Characteristics and outcomes of older adults with long-standing versus late-onset asthma. J Asthma. 2017;54(3):223-9. doi: 10.1080/02770903.2016.1211141.
3. Lockey RF. Asthma phenotypes: an approach to the diagnosis and treatment of asthma. J Allergy Clin Immunol Pract. 2014;2(6):682-5. doi: 10.1016/j.jaip.2014.09.008.

4. Bostantzoglou C, Delimpoura V, Samitas K, Zervas E, Kanniess F, Gaga M. Clinical asthma phenotypes in the real world: opportunities and challenges. Breathe (Sheff). 2015;11(3):186-93. doi: 10.1183/20734735.008115.
5. Agusti A, Barnes N, Cruz AA, Gibson PG, Heaney LG, Inoue H, et al. Moving towards a Treatable Traits model of care for the management of obstructive airways diseases. Respir Med. 2021;187:106572. doi: 10.1016/j.rmed.2021.106572.
6. Schatz M, Rosenwasser L. The allergic asthma phenotype. J Allergy Clin Immunol Pract. 2014;2(6):645-8; quiz 9. doi: 10.1016/j.jaip.2014.09.004.
7. Bakakos P. Asthma: From Phenotypes to Personalized Medicine. J Pers Med. 2022;12(11). doi: 10.3390/jpm12111853.
8. Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021;184(6):1469-85. doi: 10.1016/j.cell.2021.02.016.
9. Pongracic JA, Krouse RZ, Babineau DC, Zoratti EM, Cohen RT, Wood RA, et al. Distinguishing characteristics of difficult-to-control asthma in inner-city children and adolescents. J Allergy Clin Immunol. 2016;138(4):1030-41. doi: 10.1016/j.jaci.2016.06.059.
10. Schleich F, Maury E, Bachert C, Hanon S, Michel O, Jansen M, et al. Epidemiology of sensitization to perennial aeroallergens in adults with severe asthma in Belgium. The BEIgE study. Allergy. 2023;78(10):2774-7. doi: 10.1111/all.15785.
11. Cruz AA, Riley JH, Bansal AT, Ponte EV, Souza-Machado A, Almeida PCA, et al. Asthma similarities across ProAR (Brazil) and U-BIOPRED (Europe) adult cohorts of contrasting locations, ethnicity and socioeconomic status. Respir Med. 2020;161:105817. doi: 10.1016/j.rmed.2019.105817.
12. Beck AF, Huang B, Kercsmar CM, Guilbert TW, McLinden DJ, Lierl MB, et al. Allergen sensitization profiles in a population-based cohort of children hospitalized for asthma. Ann Am Thorac Soc. 2015;12(3):376-84. doi: 10.1513/AnnalsATS.201408-376OC.
13. Lombardi C, Savi E, Ridolo E, Passalacqua G, Canonica GW. Is allergic sensitization relevant in severe asthma? Which allergens may be culprit? World Allergy Organ J. 2017;10(1):2. doi: 10.1186/s40413-016-0138-8.
14. Tosca MA, Silvestri M, Olcese R, Pistorio A, Rossi GA, Ciprandi G. The impact of age on serum allergen-specific IgE to inhaled molecular components. Allergol Immunopathol (Madr). 2017;45(3):265-71. doi: 10.1016/j.aller.2016.10.004.
15. De Amici M, Ciprandi G. The Age Impact on Serum Total and Allergen-Specific IgE. Allergy Asthma Immunol Res. 2013;5(3):170-4. doi: 10.4168/aair.2013.5.3.170.
16. GINA. Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA) 2012. Available at: https://ginasthma.org/wp-content/uploads/2019/01/2012-GINA.pdf.
17. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.
18. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol. 2020;16(3):177-89. doi: 10.1038/s41574-019-0310-7.
19. Fernandes AGO, de Souza-Machado C, Pinheiro GP, de Oliva ST, Mota RCL, de Lima VB, et al. Dual exposure to smoking and household air pollution is associated with an increased risk of severe asthma in adults in Brazil. Clin Transl Allergy. 2018;8:48. doi: 10.1186/s13601-018-0235-6.
20. Jesus JPV, Lima-Matos AS, Almeida PCA, Lima VB, Mello LM, Souza-Machado A, et al. Obesity and asthma: clinical and laboratory characterization of a common combination. J Bras Pneumol. 2018;44(3):207-12. doi: 10.1590/S1806-37562017000000034.
21. Lima LL, Cruz CMS, Fernandes AGO, Pinheiro GP, Souza-Machado C, Lima VB, et al. Exposure to secondhand smoke among patients with asthma: a cross-sectional study. Einstein (Sao Paulo). 2020;18:eAO4781. doi: 10.31744/einstein_journal/2020AO4781.
22. Souza TMO, Fernandes JS, Santana CVN, Lessa MM, Cruz AA. Aeroallergen sensitization patterns among patients with chronic rhinitis with or without concomitant asthma. Braz J Otorhinolaryngol. 2024;90(2):101351. doi: 10.1016/j.bjorl.2023.101351.
23. Acevedo N, Caraballo L. IgE cross-reactivity between Ascaris lumbricoides and mite allergens: possible influences on allergic sensitization and asthma. Parasite Immunol. 2011;33(6):309-21. doi: 10.1111/j.1365-3024.2011.01288.x.
24. Alcantara-Neves NM, Veiga RV, Ponte JC, da Cunha SS, Simoes SM, Cruz AA, et al. Dissociation between skin test reactivity and anti-aeroallergen IgE: Determinants among urban Brazilian children. PLoS One. 2017;12(3):e0174089. doi: 10.1371/journal.pone.0174089.
25. Alcantara-Neves NM, Veiga RV, Dattoli VC, Fiaccone RL, Esquivel R, Cruz AA, et al. The effect of single and multiple infections on atopy and wheezing in children. J Allergy Clin Immunol. 2012;129(2):359-67, 367.e1-3. doi: 10.1016/j.jaci.2011.09.015.
26. Mendonca LR, Veiga RV, Dattoli VC, Figueiredo CA, Fiaccone R, Santos J, et al. Toxocara seropositivity, atopy and wheezing in children living in poor neighbourhoods in urban Latin American. PLoS Negl Trop Dis. 2012;6(11):e1886. doi: 10.1371/journal.pntd.0001886.
27. Lisboa ABP, Alcantara-Neves NM, Aguiar ERGR, Pinheiro CS, Pacheco LGC, da Silva ES. Structural Insights on Cross-Reactivity of Mite Allergens with Helminth Proteins. Allergies. 2024;4(2):64-79. doi: 10.3390/allergies4020006.
28. Hong SD, Ryu G, Seo MY, Jeong JI, Kim HY, Chung SK, et al. Optimal cutoff values of allergen-specific immunoglobulin E to house dust mites and animal dander based on skin-prick test results: Analysis in 16,209 patients with allergic rhinitis. Am J Rhinol Allergy. 2018;32(1):23-6. doi: 10.2500/ajra.2018.32.4483.
29. Lima-Matos A, Ponte EV, de Jesus JPV, Almeida PCA, Lima VB, Kwon N, et al. Eosinophilic asthma, according to a blood eosinophil criterion, is associated with disease severity and lack of control among underprivileged urban Brazilians. Respir Med. 2018;145:95-100. doi: 10.1016/j.rmed.2018.10.025.
30. Tan DJ, Walters EH, Perret JL, Lodge CJ, Lowe AJ, Matheson MC, et al. Age-of-asthma onset as a determinant of different asthma phenotypes in adults: a systematic review and meta-analysis of the literature. Expert Rev Respir Med. 2015;9(1):109-23. doi: 10.1586/17476348.2015.1000311.
31. Fernandes JS, Araujo MI, de Almeida T, Andrade LS, Lopes DM, de Mello LM, et al. Impaired immunoregulatory network of the CD4 T lymphocytes in refractory asthma. Clin Exp Allergy. 2019;49(5):644-54. doi: 10.1111/cea.13351.
32. Camelo-Nunes IC, Sole D. Cockroach allergy: risk factor for asthma severity. J Pediatr (Rio J). 2006;82(5):398-9; author reply 9-400. doi: 10.2223/JPED.1540.
33. Adham TM, Tawfik SA. Dermatophagoides in childhood asthma. Allergy to dermatophagoides associates more severe childhood asthma with a potential role for acaricides. Saudi Med J. 2012;33(3):292-7.

34. Li J, Huang Y, Lin X, Zhao D, Tan G, Wu J, et al. Influence of degree of specific allergic sensitivity on severity of rhinitis and asthma in Chinese allergic patients. Respir Res. 2011;12(1):95. doi: 10.1186/1465-9921-12-95.
35. Masaki K, Fukunaga K, Matsusaka M, Kabata H, Tanosaki T, Mochimaru T, et al. Characteristics of severe asthma with fungal sensitization. Ann Allergy Asthma Immunol. 2017;119(3):253-7. doi: 10.1016/j.anai.2017.07.008.
36. Kao CC, Hanania NA, Parulekar AD. The impact of fungal allergic sensitization on asthma. Curr Opin Pulm Med. 2021;27(1):3-8. doi: 10.1097/MCP.0000000000000740.
37. Cacheiro-Llaguno C, Mosges R, Calzada D, Gonzalez-de la Fuente S, Quintero E, Carnes J. Polysensitisation is associated with more severe symptoms: The reality of patients with allergy. Clin Exp Allergy. 2024;54(8):607-620. doi: 10.1111/cea.14486.
38. Liu AH, Babineau DC, Krouse RZ, Zoratti EM, Pongracic JA, O'Connor GT, et al. Pathways through which asthma risk factors contribute to asthma severity in inner-city children. J Allergy Clin Immunol. 2016;138(4):1042-50. doi: 10.1016/j.jaci.2016.06.060.
39. Cruz AA, Bousquet J. Rhinitis Phenotypes Based on Multimorbidities. J Allergy Clin Immunol Pract. 2024;12(6):1487-1489. doi: 10.1016/j.jaip.2024.04.013.
40. Bahceciler NN, Yuruker O. Planning and approach to allergen-specific immunotherapy in polyallergic patients. Immunotherapy. 2020;12(8):577-85. doi: 10.2217/imt-2019-0182.
41. Tran TN, Zeiger RS, Peters SP, Colice G, Newbold P, Goldman M, et al. Overlap of atopic, eosinophilic, and TH2-high asthma phenotypes in a general population with current asthma. Ann Allergy Asthma Immunol. 2016;116(1):37-42. doi: 10.1016/j.anai.2015.10.027.

EDRA ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 58, N.6, 252-262, 2026

Mira Horváthová, Jana Surmová, Nikola Madrová, Michaela Szabová

Basophil activation test in the diagnosis of drug-induced hypersensitivity reactions: a retrospective study of Slovak patients

Slovak Medical University, Faculty of Medicine, Institute of Laboratory Medicine, Polyclinic of Slovak Medical University, Department of Clinical Immunology and Allergology, Bratislava, Slovak Republic

Key words

Adverse drug reactions; basophil activation test; CD63 marker; drug hypersensitivity; flow cytometry.

Corresponding author
Mira Horváthová
Slovak Medical University
Polyclinic of Slovak Medical University
Limbová 12
833 03 Bratislava, Slovak Republic
ORCID: 0000-0002-3264-0980
E-mail: mira.horvathova@szu.sk

Doi
10.23822/EurAnnACI.1764-1489.414

Impact statement

BAT is a useful complementary tool for diagnosing DHRs, predicting and monitoring clinical responses to treatment and searching potentially non-cross-reactive alternatives.

Summary

Background. *Drug hypersensitivity reactions (DHRs) include allergic, exaggerated pharmacologic, and pseudoallergic reactions to medications. The basophil activation test (BAT) using CD63 expression is a sensitive and specific complementary tool in the diagnosis of immediate DHRs.* ***Methods.*** *In this study we analyzed retrospectively 1,160 patients with history of DHRs categorized into several subgroups of diagnoses. BAT using CD63 expression was performed in the whole blood and results were analyzed by flow cytometry.* ***Results.*** *The mean value of CD63 expression was significantly higher for positive patients than negative/borderline findings ($p < 0.001$). The highest frequencies of positive BAT were in categories of diagnosis codes D89 (other disorders involving the immune mechanism, not elsewhere classified) and L50 (urticaria). The percentage values were higher in the group of antibiotics and anticoagulants. Comparing individual drugs in vitro, we observed the highest prevalence of positive reactions by local anesthetics (Mepivastesin, Mesocain, Lidocaine), antibiotics (Amoxiclav, Megamox duo), antirheumatics, antiphlogistics and antiuratics (Ibuprofen, Ibalgin). The BAT positivity decreases with age, there were significant differences in the CD63 activation between old age and children, younger adults, and older adults ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively). No significant differences have been found between men and women.* ***Conclusions.*** *BAT is a useful complementary tool to support the diagnosis of drug hypersensitivity conditions, especially in cases with severe reaction where drug challenge is contraindicated. The implementation of BAT in clinical practice is valuable in the workup of DHRs and extremely useful in the case of life-threatening drug allergies.*

Introduction

Adverse drug reactions (ADRs) are an important public health problem and can be life-threatening. ADRs are classified into two main types: type A reactions, which are dose dependent and predictable. These kinds of reactions constitute 70-80% of ADRs, and type B – which are unpredictable – include idiosyncrasy, drug intolerance, or drug allergy, and may comprise approximately 10-15% of all ADRs. Drug hypersensitivity reactions (DHRs) are unpredictable ADRs. Depending on the time passed between the consumption of the drug and symptom onset, reactions are classified as suggestive of either immediate hypersensitivity (0-6 h) or non-immediate hypersensitivity (hours-days). Drug allergy pathogenesis and clinical manifestations may vary depending on the culprit drug. The optimal approach to the drug allergy should be based on the risk stratification, the phenotype of hypersensitivity reaction, drug class, and patient's clinical needs. Hypersensitivity reactions are divided into 4 types (I-IV) by the Gell and Coombs classifi-

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

cation. The hypersensitivity Type I reactions follow very quickly after contact with the allergen, generally between a few seconds to 30 minutes (immediate type). Allergic rhinitis and conjunctivitis, allergic asthma, acute urticaria and IgE-mediated anaphylaxis belong to this type. The response times of types II and III hypersensitivity reactions are slower than that of type I reactions; they typically develop 3-6 h after exposure to antigen. The fourth type is considered a delayed hypersensitivity reaction because it usually occurs more than 12 hours after exposure to the allergen, with a maximal reaction time between 48 and 72 hours. Type IV hypersensitivity reactions occur because of T cell response to an antigen leading to an inflammatory response. These reactions are further subdivided (IVa through IVd) based on the type of T cells involved. Common symptoms of type IV hypersensitivity include red, itchy, painful rash with blisters, or patches of dry, cracked, scaly skin. The clinical presentation is based on the distinct condition that develops. Contact dermatitis is a very common type IV hypersensitivity reaction. Symptoms without demonstration of being an immunological process are classified as non-immune DHRs, and they are generally related to nonspecific histamine release, nonspecific complement activation, bradykinin accumulation or induction of leukotriene synthesis in type I hypersensitivity reactions. Non-steroidal anti-inflammatory drugs and antibiotics are most often implicated in drug allergies, besides anesthetic drugs, latex, insulin, and immunomodulators (1-3).

The mechanisms underlying immunogenic-mediated DHRs involve the adaptive immune system and comprise both IgE- and non-IgE-mediated mechanisms. In contrast, nonimmunogenic-mediated DHRs mechanisms do not involve the adaptive immune system. The diagnosis of immune mediated DHRs is based on clinical history, immediate-reading skin tests and provocation tests. Skin prick testing (SPT) provides evidence for sensitization and can help to confirm the diagnosis of a suspected type I allergy, the intradermal test (IDT) is used for both immediate and delayed hypersensitivity and may be used when soluble forms of the drugs are available. Skin tests are the most used procedure to confirm a sensitization in drug hypersensitivity, they have high specificity, but unfortunately, the sensitivity of skin tests to most drugs is low (SPT 49% and IDT 73%, respectively) (4, 5). SPT is widely practiced, carries very low (but not negligible) risk of serious side effects to patients. The overall risk of inducing anaphylactic reactions by SPT is less than 0.02%, whereas it is slightly increased if drug, food, latex, or hymenoptera venom extracts are used. IDT has an increased antigen load to which the body is exposed through the skin, and is associated with a higher risk of systemic reaction than SPT. There are limitations to perform skin tests, *e.g.* discontinuation of some medications before testing, age, needle aversion, serious risk of anaphylaxis, skin disease. Therefore, in cases of negative reactions, drug allergies cannot be excluded. The evaluation of DHRs involves three main strategies: accurately reviewing the patient's clinical history, conducting diagnostic tests (skin tests and/or *in vitro* tests) and performing drug challenge tests. Challenge tests are currently the gold standard for diagnosis, but they often involve significant risks, especially in patients who have received multiple drugs in the context of an adverse reaction and/or patients with multiple comorbidities. In the evaluation, a careful investigation of medical history and laboratorial parameters is extremely important. *In vitro* tests can be used to support this process, and may be considered as an alternative, particularly in cases with a history of a life-threatening reaction. Tests require specialized equipment and trained personnel and thus are not broadly available. It is essential to construct networks of specialized centers to expand the knowledge of these techniques and to adequately validate them in as many centers as possible (6-10).

The basophil activation test (BAT) is a cell-based functional assay for the detection of basophils in whole blood following allergen stimulation using flow cytometry. The BAT evaluates activation markers (CD63 and/or CD203c) expressed on the cell membrane of basophils. CD63 is a membrane-bound molecule, intragranularly expressed, and exposed on the cell surface during degranulation where granular membrane fuses with the cellular membrane. Basophils constitute a minor fraction – less than 1% of the circulating white blood cells – and upon IgE cross-linking by antigen, can activate and degranulate. They expel the preformed content from their granules, as well as *de novo* synthesized mediators. Degranulation of basophils can also result from other mechanisms, such as non-IgE-mediated pathways (*e.g.* cytokines, anaphylatoxins, IgG) and/or direct activation (*e.g.* opioids, iodinate contrast media). BAT could be a predictive test evaluating CD63 as a biomarker of basophil activation for the assessment of immediate DHRs. BAT is more sensitive and specific than other *in vitro* diagnostic techniques in drug allergy and is essentially complementary to skin tests and allergen-specific IgE determinations. Increasing the number of diagnostic tests used to confirm a suspected clinical history of allergy can improve diagnosis efficiency and accuracy. BAT reduces the need for *in vivo* procedures, such as intradermal tests and allergen challenges, which can cause allergic reactions of unpredictable severity. It is a very important diagnostic tool, less invasive, more comfortable and less expensive. The main objective in the management of a DHRs is to search for the culprit drug to order instruct future avoidance, when possible, and to clarify tolerance to alternative treatments for the patient (11-13).

The aim of this research is to explore retrospectively a cohort of patients recruited from 2020 to 2023 at diagnostic Immunology Laboratory of Slovak Medical University in Bratislava and tested to DHRs. We evaluated BAT, the expression of activation marker CD63, and confounders such as age, gender, diagnostic category code and drug class. BAT not only complement culprit identification but can also be used to test potentially non-cross-reactive alternatives, thereby avoiding drug challenge tests.

Materials and methods

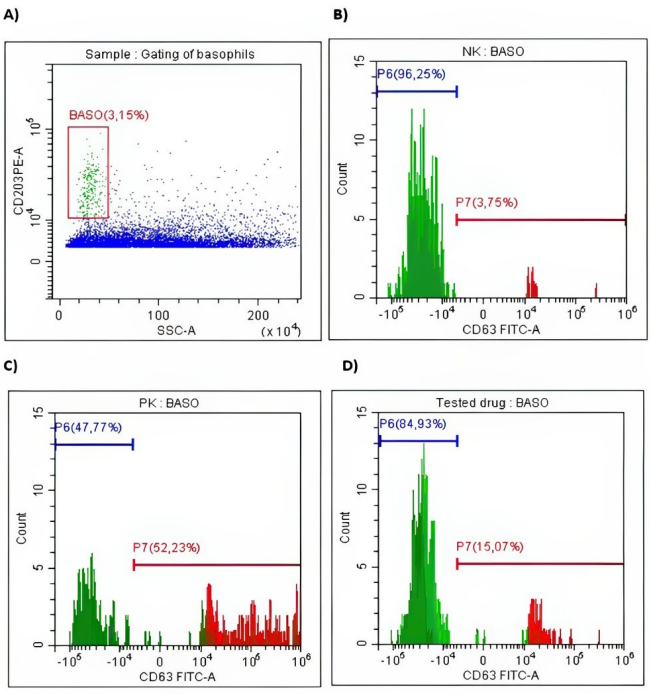
Study population

We performed a cohort study assessing DHRs on collected clinical data from 1,160 patients (876 women and 284 men). Some patients were tested *in vitro* for more than one type of drug. A total of 3,223 laboratory tests were evaluated. The study was retrospective and did not require any interaction with the patients. Ethical approval was waived by the local Ethics Committee of the Slovak Medical University in view of the retrospective nature of the study and all the procedures being performed were part of the routine care. The participants were referred to Polyclinic of Slovak Medical University (SMU Polyclinic) after detailed anamnesis and allergological workup performed by clinical allergists/immunologists and dermatologists from Bratislava and surroundings over the years 2020 to 2023. The blood sampling was provided in the collection room of SMU Polyclinic and BATs were processed in the diagnostic Immunology Laboratory. The patients were instructed to stop taking antihistamines, antileukotrienes and glucocorticoids at least 48 hours before sampling. Antihistamines and antileukotrienes do not interfere with BAT results, the oral administration of these pharmacological substances may affect others cell function assays that we performed (*e.g.* lymphocyte activation and proliferation – submitted). Blood samples were drawn from patients at least 2-4 weeks post-hypersensitivity reaction, collection of whole blood was done in heparin and performed within 4 h of blood collection to maximize viability and functionality of basophils.

Basophil activation test (BAT)

For the quantification of immediate DHRs was used BAT, that analyses expression of basophil activation marker, CD63. Our study was focused on the basophil population at a single cell level using flow cytometry. This technique allowed the specific examination of basophils in whole blood, that is more physiological and more closely resemble the *in vivo* environment of blood basophils, than isolated basophils. Heparinized blood was incubated with or without relevant drugs. The optimization procedure consisted of standardization of drug concentrations, using two drug concentrations, dilutions were prepared shortly before BAT was performed. Analysis of the CD63 antigen on basophils was examined using the BasoFlowEx kit (Exbio, Prague, Czech Republic) with a modification for the lyse-no-wash procedure (14). Briefly, tubes were prepared for negative control, positive control (stimulation control), and samples stimulated with different drug substances. We tested the common commercially available drugs used in therapy. The pharmaceuticals were in the form of tablets, or injectable preparations. Stock solutions were produced by pulverizing if necessary and dissolving the drug in 10 ml of sterile isotonic saline (NaCl 0.9%), after checking concentration of drug solubility in aqueous solutions. The concentrations of stock solutions were based on active substance content of the concerned medicine. In the test, we used two drug dilutions, or more as needed, to find conditions in which cell activation and survival outweigh cell death. For example, the range of final concentration was 0.25-2.5 mg/ml (Amoxicillin and Clavulanic Acid), 0.2-2.0 mg/ml (Metamizole sodium salt, Paracetamol), 0.15-1.5 mg/ml (Ibuprofen), 0.1-1.0 mg/ml (Mepivacaine hydrochloride), 0.08-0.8 (Lidocaine hydrochloride), 0.04-0.4 mg/ml (Trimecaine hydrochloride, Propofol), 0.45-4.5 mg/ml (Nadroparin calcium salt). For hypolipidemic category (*e.g.* Atorvastatin, Simvastatin, Fluvastatin) the range 0.01-0.3 mg/ml was used. Stimulation Buffer and heparinized blood were added into all tubes and incubated at 37 °C for 15 minutes. Staining reagent (antibody cocktail: anti-CD63-FITC + anti-CD203c-PE) was added, and samples were incubated for 20 minutes at 2-8 °C. The red blood cells were lysed by lysing solution OptiLyse C (Beckman Coulter, Marseille, France) and samples were analyzed using clinical flow cytometers (Cytomic FC500 and DxFLEX, Beckman Coulter) within two hours after staining. Enough basophils for analysis were > 200. Histograms of negative control and positive control samples, and drug-stim-

Figure 1 *- Example of BAT histograms (negative control, positive control, and drug-stimulated basophils).*



BAT: basophil activation test. **(A)** Gating of basophils: basophils were identified as CD203+ cells; **(B)** Negative control sample, CD63 negative threshold (P7 = 3.75 %); **(C)** Positive control sample, value of ≥ 15% activated basophils (P7 = 52.23 %); **(D)** Drug-stimulated sample, CD63 positivity: the cut-off value ≥ 5% and stimulation index (SI) ≥ 2 (P7 = 15.07 %).

ulated basophils are shown in **figure 1**. We have calculated the percentage of activated basophils (CD63+), and individuals are considered positive when the percentage of CD63+ exceeds the cut-off value ≥ 5% and stimulation index (SI) ≥ 2. The limit of borderline range (suspect zone) was < 5% and SI ≥ 2. If the positive control sample exhibited a value of ≥ 15 activated basophils, the samples could be evaluated. A positive control sample was stimulated using a monoclonal antibody against IgE molecule which mimics the allergen crosslinking process of IgE molecules on the basophil surface and using a chemotactic peptide N-formyl-Met-Leu-Phe (fMLP). The fMLP activates basophils through the G-protein coupled fMLP receptors and is often used as a non-IgE-mediated positive control. The main limitation of BAT was the patient with nonresponder basophils (basophils that do not respond to stimulants), rendering BAT uninterpretable. Nonresponder and patients with low basophil counts were excluded from the study (6%). Non-responsiveness is attributed to disturbances in the signalosome of the FcεRI pathway, particularly failure to express the downstream tyrosine kinase Syk (15, 16). In case of positive BAT, it is not necessary to perform a provocation test. Negative results could not rule out a suspected drug, they may help the allergist manage and ensure availability of appropriate drugs for further *in vivo* test or drug provocation test. A no-positive answer indicates that patients may not actually be allergic to the suspected drug or have the chance to use alternative drugs.

Statistical analysis

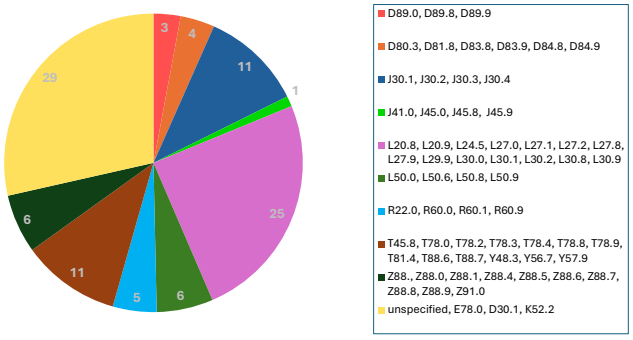
BAT data was tested for normalized distribution and analyzed using the Mann-Whitney test. The expression of CD63 was described as median (50th percentile), and statistical significance was defined $p < 0.05$. The median was calculated using descriptive statistics. All statistical analyses were conducted using SPSS Statistics, version 27 (IBM, USA). The subjects with unresponsive basophils were eliminated from data analysis.

Results

Subjects were referred to diagnostic testing after experiencing a drug-induced hypersensitivity reaction. The time elapsed between the reaction and the performance of the test differed in patients, on average 3-5 months. The most common clinical symptoms were skin reaction, pruritus, rash, urticaria, redness, vomiting, heart palpitations, swelling of the face, tongue, difficulty breathing and swallowing, anaphylactic reaction, collapse state. Detailed and complete description of clinical symptoms by doctors not stated for every patient. Results of skin prick tests or specific IgE were not available for those who performed BAT. Patients were divided into several groups based on clinical symptoms (*e.g.* cutaneous, respiratory, gastrointestinal, cardiovascular, hypotension, confusion), in which we monitored changes in CD63 expression and frequency of BAT positivity.

We most often tested the diagnoses indicated by the codes of International Classification of Diseases (ICD) (17): L20-L30 (dermatitis and eczema), J30 (vasomotor and allergic rhinitis), T78 (adverse effect, not elsewhere classified), Z88 (personal history of allergy to drugs, medicaments and biological substances), L50 (urticaria) and group of patients with an unspecified diagnosis. To process the results, we categorized the probands into ten subgroups of diagnoses (**figure 2**). The group of dermatitis and eczema (25% of the total) includes subgroups: L20.8 - other atopic dermatitis, L20.9 - atopic dermatitis, unspecified, L24.5 - irritant contact dermatitis due to other chemical products, L27.0 - generalized skin eruption due to drugs and medicaments, L27.1 - localized skin eruption due to drugs and medicaments, L27.2 - dermatitis due to ingested food, L27.8 - dermatitis due to other substances taken internally, L27.9 - dermatitis due to unspecified

Figure 2 *- Percentage distribution of patients according to subgroups of diagnoses (ICD codes).*



ICD: International Classification of Diseases; World Health Organization (2019). International statistical classification of diseases and related health problems (11th ed.). Available at: https://icd.who.int/.

D89 - Other disorders involving the immune mechanism, not elsewhere classified. D80 - Immunodeficiency with predominant antibody defects. D81 - Combined immunodeficiencies. D83 - Common variable immunodeficiency. D84 - Other immunodeficiencies. J30 - Vasomotor and allergic rhinitis. J41 - Simple and mucopurulent chronic bronchitis. J45 – Asthma. L20 - Atopic dermatitis. L24 - Irritant contact dermatitis. L27 - Dermatitis due to substances taken internally. L29 – Pruritus. L30 - Other dermatitis. L50 - Urticaria. R22 - Localized swelling, mass and lump of skin and subcutaneous tissue. R60 - Edema, not elsewhere classified. T45 - Poisoning by primarily systemic and hematological agents, not elsewhere classified. T78 - Adverse effects, not elsewhere classified.

T81 - Complications of procedures, not elsewhere classified. T88 - Other complications of surgical and medical care, not elsewhere classified. Y48 - Anesthetics and therapeutic gases. Y56 - Topical agents primarily affecting skin and mucous membrane and ophthalmological, otorhinolaryngological and dental drugs. Y57 - Other and unspecified drugs and medicaments. Z88 - Personal history of allergy to drugs, medicaments and biological substances. Z91 - Personal history of risk-factors, not elsewhere classified. E78 - Disorders of lipoprotein metabolism and other lipidemias. D30 - Benign neoplasm of urinary organs. K52 - Other noninfective gastroenteritis and colitis.

substance taken internally, L29.9 - pruritus, unspecified, L30.0 - nummular dermatitis, L30.1 - dyshidrosis (pompholyx), L30.2 - cutaneous autosensitization, L30.8 - other specified dermatitis, L30.9 - dermatitis, unspecified. The group of vasomotor and allergic rhinitis (11 % of the total) included J30.1 - allergic rhinitis due to pollen), J30.2 - other seasonal allergic rhinitis, J30.3 - other allergic rhinitis, J30.4 - allergic rhinitis, unspecified. The group of patients with lower airway diseases J41.0 - simple chronic bronchitis, J45.0 - predominantly allergic asthma, J45.8 - mixed asthma, J45.9 - asthma, unspecified was represented by only 1%. The age distribution of patients was children (1-17 years; 8%), younger adults (18-44 years; 29%), older adults (45-64 years; 36%), and old age (65-99 years; 27%).

We compared the CD63 marker obtained by flow cytometry analysis between groups: a) negative response or borderline (suspect zone) and b) positive response to a drug. The distribution data were represented as percentile values (Q1 = 5.9; Q2 = 7.8; Q3 = 13.2). The median value of CD63 expression in positive BAT response was significantly higher than in negative or borderline response (1.60 %) ($p < 0.001$). Of the total number of BAT, we recorded about 10% positive drug responses (n = 3,223). BAT is expected to be positive for reactions that are clinically type I, this functional test measures the degree of basophil degranulation following allergen stimulation. In the case of BAT negativity, there may be another type of hypersensitivity reaction, a false negativity, or another mechanism, such as non-IgE mediated or direct basophil activation.

The frequency of BAT positivity decreases with age. The prevalence of positive drug responses was as follows: children 23.4% of 154 tests, younger adults 10.4% of 838 tests, older adults 9.2% of 1,230 tests, and the elderly 7.6% of 1,001 tests. We did not observe significant differences in the response to stimulants in the positive control between age groups. The difference in frequency of positive basophil activation between men (11.8% of 738 tests) and women (9.1% of 2,485 tests) was not significant. Patients with diagnoses a) D89.0 (polyclonal hypergammaglobulinemia), D89.8 (other specified disorders involving the immune mechanism, not elsewhere), D89.9 (disorder involving the immune mechanism, unspecified), and b) L50.0 (allergic urticaria), L50.6 (contact urticaria), L50.8 (other urticaria), L50.9 (urticaria, unspecified) represented the highest frequency of positive BAT (13.8% of 94 tests, and 12.6% of 167 tests, respectively) (**table I**).

The most frequently tested drug classes were anesthetics and antibiotics, as shown in **figure 3**. The highest frequency of BAT positivity was for antibiotics (16.2% of 464 tests) and anticoag-

Table I *- Frequency of BAT positive responses.*

Diagnosis (ICD codes classification)	Frequency of BAT positivity (%)	Total number of BAT
D89 - Other disorders involving the immune mechanism, not elsewhere classified	**13.8**	94
D80 - Immunodeficiency with predominantly antibody defects, D81 - Combined immunodeficiencies, D83 - Common variable immunodeficiency, D84 - Other immunodeficiencies	7.3	96
J30 - Vasomotor and allergic rhinitis	9.5	380
J41 - Simple and mucopurulent chronic bronchitis, J45 – Asthma	11.4	44
L20 - Atopic dermatitis, L24 - Irritant contact dermatitis, L27 - Dermatitis due to substances taken internally, L29 - Pruritus, L30 - Other dermatitis,	9.1	821
L50 - Urticaria	**12.6**	167
R22 - Localized swelling, mass and lump of skin and subcutaneous tissue, R60 - Edema, not elsewhere classified	10.1	159
T45 - Poisoning by primarily systemic and hematological agents, not elsewhere classified, T78 - Adverse effects, not elsewhere classified, T81 - Complications of procedures, not elsewhere classified, T88 - Other complications of surgical and medical care, not elsewhere classified, Y48 - Anesthetics and therapeutic gases, Y56 - Topical agents primarily affecting skin and mucous membrane and ophthalmological, otorhinolaryngological and dental drugs,Y57 - Other and unspecified drugs and medicaments	9.8	328
Z88 - Personal history of allergy to drugs, medicaments and biological substances, Z91 - Personal history of risk-factors, not elsewhere classified	6.2	210
E78 - Disorders of lipoprotein metabolism and other lipidemias, D30 - Benign neoplasm of urinary organs, K52 - Other noninfective gastroenteritis and colitis	10.2	924

Values in bold indicate the highest frequencies of positive BAT responses, which are further detailed in the text. ICD: International Classification of Diseases. World Health Organization (2019). International statistical classification of diseases and related health problems (11th ed.). Available at: https://icd.who.int/.

Figure 3 *- Drug classes frequently tested using BAT (%) (minimum number of laboratory tests ≥ 5%).*

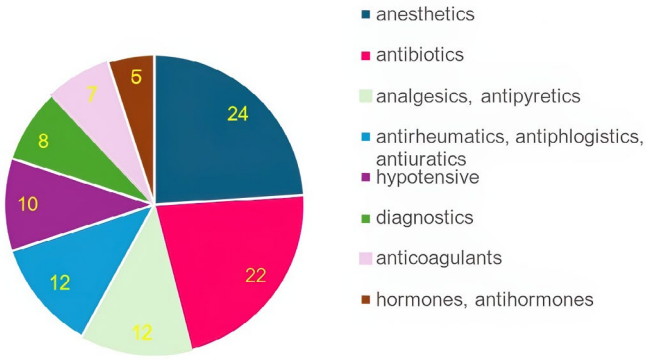
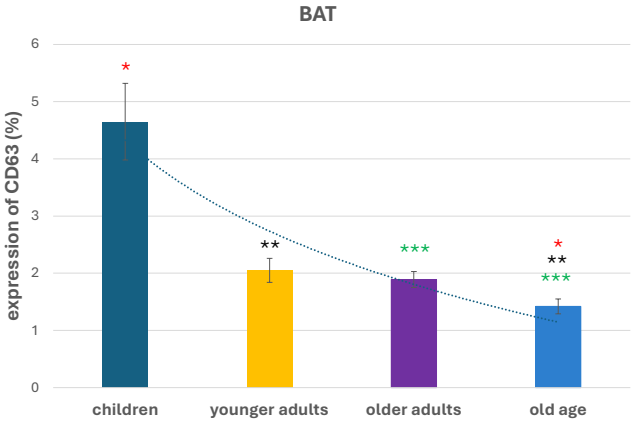


Figure 4 *- Distribution of CD63 expression in different age groups.*



BAT: basophil activation test. *Between old age and children, $p < 0.001$; **between old age and younger adults, $p < 0.05$; ***between old age and older adults, $p < 0.01$.

ulants (12.6% of 143 tests). Negative tests predominated in the classes of diagnostics (87.3% of 158 tests), hypotension (85.3% of 204 tests) and anesthetics (83.3% of 491). By comparing individual drugs, we found that the most frequent positivity of BAT *in vitro* belongs to 1) the antibiotics Amoxiclav, Megamox duo (Amoxicillin and Clavulanic acid), 2) among the local anesthetics Mepivastesin (Mepivacaine hydrochloride), Mesocain (Trimecaine hydrochloride), Lidocaine (Lidocaine hydrochloride), 3) the general anesthetic Propofol), 4) the class of antirheumatic drugs, antiphlogistics and antiuratics Ibalgin (Ibuprofen) and Ibuprofen, 5) anticoagulant Fraxiparine (Nadroparin calcium salt), and 6) analgesics, antipyretics Novalgin (Metamizole sodium salt) and Paralen (Paracetamol) (**table II**).

We recorded a positive response to at least two drugs in 4.5% of the total number of patients, 2% were drugs of the same group (especially antibiotics) and 0.25 % were drugs with the same substance.

Children, younger adults, and older adults had a high frequency of positive BAT responses to antibiotics. In old age, the positivity of hypolipidemic drugs dominated, but the drug representation for this category of drugs was very low (< 5% of total BAT). There were significant differences in CD63 expression between the elderly and other groups, namely children, younger adults, and older adults ($p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively) (**figure 4**). CD63 expression was not significantly different between men and women.

The patients were analyzed also according to presenting symptoms, most of them presented more symptoms. There were no differences between clinical manifestations of drug allergies (*e.g.* cutaneous, respiratory, gastrointestinal, cardiovascular, hypotension) and CD63 marker expression or BAT positivity frequency. No significant differences were found when comparing the frequency of positive BAT between patients with and without anaphylactic reaction.

Table II *- Drugs with the most frequent BAT positivity.*

Commertial name of drug	Effective drug substance	Drug class
Amoxiclav, Amoxixlav syrup, Megamox duo	Amoxicillin and Clavulanic acid	Antibiotics
Mepivastesin	Mepivacaine hydrochloride	Local anesthetics
Mesocain	Trimecaine hydrochloride	Local anesthetics
Lidocain	Lidocaine hydrochloride	Local anesthetics
Propofol	Propofol	General anesthetics
Ibuprofen, Ibalgin	Ibuprofen	Antirheumatics, antiphlogistics, antiuratics
Fraxiparine	Nadroparin calcium salt	Anticoagulants
Novalgin	Metamizole sodium salt	Analgesics, antipyretics
Paralen	Paracetamol	Analgesics, antipyretics

Discussion and conclusions

DHRs induced by small molecule drugs include a broad spectrum of ADRs with heterogeneous clinical presentations and mechanisms. A high number of unconfirmed and/or self-reported DHRs is a frequent problem in daily clinical practice with impact on future prescription choices and patient health. The *in vitro* BAT can serve as a quick, reliable, and safe diagnostic tool, that can provide information about diagnostic algorithms for the management of DHRs. The utility of BAT is in patients with immediate DHRs, whom *in vivo* testing was considered high risk or when commercial antigens were not available (18, 19).

Assays which assess the activation of basophils by the changes in cell surface markers (CD63, CD203c) are now in routine use. CD203c has been identified as specific for basophils and is expressed on resting cells at low levels, and its expression is rapidly up-regulated following activation. The activation is transient and more rapid than expression of CD63, so assays which use only CD203c require careful consideration of the timing for detection, but the selectivity of this marker for basophils (among all circulating leukocytes) make this an attractive marker of activation (20). Several factors may influence the quality of the results, these include sampling conditions, relevant allergens, basophil-gating strategies, markers for detection of activated cells, drug concentrations, time that elapses between the reaction and blood sampling, medications used by the patient who is being tested. It is important for the clinician to understand the potential promise of *in vitro* tests for confirming or ruling out a diagnosis of DHRs. A positive response will lead to an increase in the probability that a diagnosis of DHRs is present and to subsequent drug avoidance. A negative test response should reduce the probability of an association between the test drug and DHRs and preclude avoidance of the suspect drug or further diagnostic testing. There is a need for characterization of a larger numbers of exposed control subjects (21, 22). DHRs are a burden for patients and health systems, not only due to the increasing prevalence (10-20% of hospitalized patients and up to 25 % of outpatients), but also to the complexity and severity of the reactions (23). Our study confirmed 1/10 of positive results from the total numbers of laboratory tests, CD63 expression in positive patients was significantly higher than in negative and/or borderline (suspicious) responses. Currently, several endophenotypic categories of DHRs are defined, such as type I IgE/non-IgE, cytokine release, mast-related G-protein coupled receptor X2 and cyclooxygenase-1 (COX-1). A proper knowledge of endotypes based on specific biomarkers will permit discriminating patients within the same phenotype. For diagnostic purposes, the fact that positive basophil responses cannot distinguish between sIgE-FcεRI cross-linking and alternative mechanisms is not a disadvantage. BAT can help to deepen our understandings in mechanistic endotypes of immediate DHRs, benefit identification of antibody recognition sites, expand our understanding of desensitization and tolerance induction strategies, and predict natural disease courses and prognosis. The mechanisms responsible for the immediate DHRs, without prior exposure and with low opportunity for prior sensitization to the drug, may raise hypothesis of non-IgE-mediated pathways, such as complement activation with release of anaphylatoxins, or direct activation of basophils and mast cells (24-26).

Anesthetics and antibiotics are the most common classes of drug tested in our laboratory. When comparing different drugs, we observed the highest frequency of positive results with antibiotics Amoxiclav, Megamox duo (Amoxicillin, Clavulanic acid), among local anesthetics Mepivastesin (Mepivacaine hydrochloride), Mesocain (Trimecain), Lidocaine (Lidocaine chloride monohydrate). Positive reactions *in vitro* also include general anesthetic Propofol, anticoagulant Fraxiparine (calcium salt of Nadroparin), antirheumatic, antiphlogistic and antiuratic drugs (Ibuprofen, Ibalgin - Ibuprofen) and analgesics, antipyretics (Novalgin - Metamizole sodium, Paralen - Paracetamol).

Most studies from allergy centers and clinics reported that the common culprit drugs among DHRs were antibiotics, radiocontrast media and antineoplastic drugs. Drug-specific cofactors for DHRs are diseases such as obesity and atopic dermatitis, knowledge of which may improve risk calculation in drug challenge trials (27-30). Amoxiclav is currently the most frequently reported beta-lactam in Europe. Another component, the Clavulanic acid, has been recently identified as prevalent triggers in perioperative anaphylaxis. The high positive predictive value of BAT to clavulanic acid shows its potential value as a complementary tool to the allergological workup of patients with immediate allergic reactions after amoxicillin-clavulanic acid treatment. Importantly, the assay should be done within the first 12 months after the reaction to reduce false-negative results (31-34).

The immediate allergic reactions to amide local anesthetics are considered very rare. However, the frequency of allergic reactions to them has recently increased, probably due to the preferential use of these anesthetics. The formulation of propofol (general anesthetic) contains other ingredients, such as refined soybean oil, medium chain triacylglycerols, injectable egg phospholipids, glycerol, and sodium oleate. Drug hypersensitivity in *in vitro* test might be to propofol or to one of the other components of the composition of the commercial preparation (35, 36). Zuo *et al.* (37) investigated the risk of true allergy to local anesthetics, they introduced only 6 from 68 patients with a suspected DHRs to local anesthetics and demonstrated positive results in skin tests and/or BAT. The authors conclude that skin tests and BAT may be reliable methods for investigation and diagnosis of true allergies to local anesthetics in clinical practice.

Anticoagulants belong to the most widely used drugs, all of them may provoke hypersensitivity reactions based on various pathogenetic pathways, different clinical manifestations and degrees of severity. We found a high percentage of positive BAT with these

drugs. Hypersensitivity reactions were formerly attributed to preservatives and contaminants, such as proteins of animal origin. Low molecular weight heparins cause especially a delayed-type, non-IgE-mediated hypersensitivity response. Among various types of skin reactions, immediate hypersensitivity reactions may be caused by chondroitin sulphate of the incomplete heparin purification. Nadroparin calcium is a heterogeneous combination of sulphated polysaccharide glycosaminoglycan chains. Heparins may possibly bind to multiple cell surface proteins (38-40). Previous studies have shown that heparin promotes both *de-novo* generation and activation of T regulatory (Treg) cells, which do not suppress rather induce activation of basophils (41, 42). The experimental results indicated that heparin may potentiate CD63 expression, potentially influencing exosome functions. Heparin may increase white blood cell counts and can bind a variety of signaling molecules such as growth factors, cell surface proteins of pathogens and most notably, cell adhesion molecules. These signaling molecules are involved in cell communication, acting as ligands, receptors and second messengers. The noncovalent interactions of sulphated polysaccharides with proteins effect changes in protein conformation, facilitate protein-protein interaction, and sequester proteins at the cell surface. Long-term nadroparin calcium injections can cause localized and generalized cutaneous ADRs, an increasing number of cutaneous ADRs have been reported (43-47). It is speculated that the hypersensitivity to nadroparin calcium may be related to its special antigenic determinants. Ebo *et al*. (48) presented a case of combined immediate and delayed type of hypersensitivity reaction to nadroparin calcium in a patient tolerant for unfractionated heparins and dalteparin. Ibuprofen is a commonly used antipyretic and analgesic nonsteroidal anti-inflammatory drug (NSAID). The ingestion of lipid transfer proteins (plant-food allergens) alongside NSAIDs (commonly ibuprofen) can trigger anaphylaxis, urticaria, and angioedema. ADRs to NSAIDs can be caused by specific immunological mechanisms (allergic reactions) or by biochemical processes linked to arachidonic acid metabolism, *e.g*. nonallergic hypersensitivity or cross-intolerance reactions. The precise patient phenotyping and pharmacogenetics information will improve the understanding and management of DHRs (49, 50). BAT, based on the detection of CD63 upregulation induced by NSAIDs, has been described. Positive tests were more frequent among patients having a severe hypersensitivity (grade II) contrasting to patients suffering from NSAID hypersensitivity restricted to cutaneous reaction (grade I). BAT is useful for the *in vitro* diagnosis of NSAID hypersensitivity, providing good specificity and positive predictive value and diagnostic reliability in the assessment of NSAID intolerance (51, 52). Metamizole belongs to the category of non-opioid analgesics, and as for other NSAIDs such as acetylsalicylic acid, diclofenac, or ibuprofen, both isoforms of cyclooxygenase (COX-1 and COX-2) are inhibited. IgE-mediated allergic reactions are found in only a small proportion of patients suffering from early onset reactions (53, 54). BAT has a good correlation with skin test results to metamizole and may be a useful diagnostic tool in patients with severe immediate allergic reactions to pyrazolones (55).

Paracetamol allergy is not common, and many of the reactions are related to the pharmacological action of COX-1 inhibition. Selective and IgE-mediated hypersensitivity reactions are rare. Cross-intolerance to NSAIDs was observed among 25% of patients with paracetamol hypersensitivity (56, 57). Bergeret *et al*. (58) reported a rare case of immediate allergic hypersensitivity to paracetamol with positive intradermal test and BAT. The authors concluded that BAT correlated with the relevant clinical history and can be a reliable tool to identify the culprit drug. We pointed out that antibiotics dominated in children, younger adults and older adults, in old age a positive response to hypolipidemics prevailed. Our data contrast with Accarino *et al*. (59) who show that the number of reported allergies to antibiotics increases with age, and that older adults have an increased risk of negative effects associated with alternative antibiotic use. BAT is an advantage from a clinical point of view, especially for a geriatric patients, who are often affected by multiple diseases.

Statins exhibit pleiotropic effects potentially affecting several immune response properties including immune cell activation, migration, cytokine generation, immune metabolism, and survival. They are derived from two origins: biological – extracted from *Penicillium* and *Aspergillus* fungal metabolites (*e.g*. Simvastatin), and synthetic (*e.g*. Atorvastatin, Fluvastatin, Rosuvastatin), and may upregulate proinflammatory cytokine production (60, 61). Kolawole *et al*. (62) demonstrated that Fluvastatin modulate basophils degranulation *in vitro*. Simvastatin was found to modulate the secretion of exosome from various cell types, CD63 decreased in dose-dependent manner (63).

We recorded a very low amount of positivity for hypolipidemic drugs; however, a higher frequency of positive response was detected in old age group. The CD63 and CD81 markers have been identified as indicators of biological aging. Exosomes play a significant role in cellular communication and significantly impact the aging process. Apoptosis is involved in aging and age-related diseases. In addition to inhibiting proliferation, Simvastatin was also found to promote cell cycle arrest and induce apoptosis. Statins primed apoptosis through its intrinsic pathway involving the mitochondria, the apoptotic pathway was presumably regulated by altered prenylation of cell signal transduction such as Ras and RhoA. The selective analysis of apoptotic *versus* nonapoptotic cells proved that both the increased expression of the tetraspanin CD63 and the loss of CD62L were restricted to the apoptotic subpopulation (64-69). Our study confirms a decreasing trend with age in the frequency of BAT positivity. Elderly patients had 7.6% positive responses and significantly differed in CD63 expression from all other groups, showing reduced expression of the receptor. DHRs may be less frequent and/or less severe in elderly possibly due to the involution of the immune system typical of this period of life. Ven-

tura *et al.* (70) report a rate of DHRs in the elderly of 30%, and the much higher activity explain by the fact that many affected patients were not referred for examination by a specialist. Polypharmacy is more common in the geriatric population as they suffer from multiple co-morbidities. Bielen *et al.* (71) have demonstrated that drug hypersensitivity was most common in young women aged 18-29 years, and the incidence of suspected drug hypersensitivity lowered with increasing age.

We found no gender differences in CD63 expression, in agreement with Koumaki *et al.* (22). We established the highest frequency of BAT positivity in some groups classified according to the ICD, D89 (other disorders involving the immune mechanism, not elsewhere classified) and L50 (urticaria). Mechanisms triggering the pathogenesis of chronic spontaneous urticaria (CSU) have been identified as type I autoallergic (which is associated with IgE antibodies against autoantigens) and type IIb autoimmune (which is driven by autoantibodies to FceR1 and/or IgE). NSAIDs have been found to exacerbate urticaria (NSAID-exacerbated urticaria) in 10% to 30% of patients with CSU (72, 73). Netchiporouk *et al.* (74) show that positive CD63 BAT are common in autoimmune CSU and are associated with high disease activity, children with CSU showed significantly increased BAT values compared to healthy controls.

Some controversies about classification of DHRs persist due to overlapping reactions and the appearance of similar symptoms, which may be caused by quite different immune and even non-immune mechanisms. We observed no differences between clinical manifestations of drug allergies (*e.g.* cutaneous, respiratory, gastrointestinal, cardiovascular, hypotension, confusion) and the activation marker CD63 and/or frequency of BAT positivity (data not shown). ADRs represent a major impact on society, increase morbidity, mortality, and healthcare costs. Considerable progress has been made in understanding the mechanisms of DRs, the physicochemical properties of drugs, the identification of risk compounds, and the specific characteristics of patient populations (75).

In conclusion, BAT is useful tool for diagnosing DHRs, predicting and monitoring clinical responses to treatment. This *in vitro* flow cytometric method eliminates the need for *in vivo* procedures that can cause unpredictable and severe reactions. BAT is a rapid and safe diagnostic tool for patients with drug-induced anaphylaxis. Further research is needed to standardize drug allergy phenotypes, endotypes and biomarkers.

Fundings

None.

Contributions

MH, MS: conceptualization. JS, NM, MS: formal analysis, investigation. MH, MS: methodology. NM, JS: resources. MH, MS: supervision. MH: writing – original draft. MH, MS, NM, JS: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

The authors thank the nurses and doctors specializing in immunology, allergology and dermatology, who participated in the study, including the staff of the SMU Polyclinic.

References

1. Nagao-Dias AT, Teixeira, FM, Coelho, HLL. Diagnosing immune-mediated reactions to drugs. Allergol Immunopathol. 2009;37(2):98-104. doi: 10.1016/s0301-0546(09)71112-7.
2. Copaescu AM, Kimberly LL, Blumenthal G, Trubiano JA. How to define and manage low-risk drug allergy labels. J Allergy Clin Immunol Pract. 2024;12(5):1095-106. doi: 10.1016/j.jaip.2024.03.021.
3. Gentry Wilkerson R. Drug Hypersensitivity Reactions. Emerg Med Clin N Am. 2022;40:39–55. doi: 10.1016/j.emc.2021.09.001.
4. Saff RR. Skin testing as a biomarker in drug allergy. Ann Allergy Asthma Immunol. 2023;130(2):161-168. doi: 10.1016/j.anai.2022.10.006.
5. Singh SK, Bohara A, Rai Tulica. Comparison of Sensitivities of Skin Prick and Intradermal Tests with Oral Rechallenge Test: A Prospective Interventional Hospital-Based Study. Indian. J Dermatol. 2021;66(1):55-59. doi: 10.4103/ijd.IJD_554_19.
6. Tanno LK, Darlenski R, Sánchez-Garcia S, Bonini M, Vereda A, Kolkhir P, et al. International survey on skin patch test procedures, attitudes and interpretation. World Allergy Organ J. 2016;9:8. doi: 10.1186/s40413-016-0098-z.
7. Sabaté-Brescó M, Quan PL, Goikoetxea MJ. Optimising the utility of in vitro tests for the diagnosis of drug allergy: Insights from a clinical perspective. Curr Treat Options Allergy. 2023;10(3):267-282. doi: 10.1007/s40521-023-00345-6.
8. AlGassim M, Alawami F, Bakhsh GH, Al-Shouli S, Alhumaid NK, Abdullah A, et al. Towards standardized skin testing for drug-induced allergic reactions. Saudi Pharm J. 2025;33(4):26. doi: 10.1007/s44446-025-00031-5.
9. Carvalho J, Oliveira G. Systemic reaction during intradermal skin tests with beta-lactams. BMJ Case Rep. 2021;14(3):e240050. doi: 10.1136/bcr-2020-240050.
10. Ene-Cociș M, Mureșan M, Petrișor C, Hagău, N, Onițiu-Gherman N. Incidence of systemic reactions to skin tests and drug challenge tests. Revue Française d'Allergologie. 2018;58(5):367-372, doi: 10.1016/j.reval.2018.03.001.
11. Alvarez-Arango S, Kumar M, Chow TG, Sabato V. Non-IgE-mediated immediate drug-induced hypersensitivity reactions. J Allergy Clin Immunol Pract. 2024;12(5):1109-1119. doi: 10.1016/j.jaip.2024.02.019.
12. Steiner M, Harrer A, Himly M. Basophil Reactivity as Biomarker in Immediate Drug Hypersensitivity Reactions - Potential and Limitations. Front Pharmacol. 2016;7(171):1-12. doi: 10.3389/fphar.2016.00171.
13. Campos L, Galvão VR, Kalil J, Castells M, Giavina-Bianchi P. BAT in the Diagnosis of Drug Allergy: A Novel Tool in Clinical Daily

Practice? Curr Allergy Asthma Rep. 2019;19(4):20. doi: 10.1007/s11882-019-0852-8.
14. Srinoulprasert Y, Rerkpattanapipat T, Sompornrattanaphan M, Wongsa C, Kanistanon D. Clinical value of in vitro tests for the management of severe drug hypersensitivity reactions. Asia Pac Allergy. 2020;10(4):e44. doi: 10.5415/apallergy.2020.10.e44.
15. Ebo DG, Bridts CH, Mertens CH, Sabato V. Principles, potential, and limitations of ex vivo basophil activation by flow cytometry in allergology: A narrative review. J Allergy Clin Immunol. 2021;147:1143-53. doi: 10.1016/j.jaci.2020.10.027.
16. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice. Allergy. 2021;76(8):2420-2432. doi: 10.1111/all.14747.
17. World Health Organization. International statistical classification of diseases and related health problems (11th ed.). 2019. Available at: https://icd.who.int/.
18. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy. 2015;70(11):1393-1405. doi:10.1111/all.12698.
19. Kim SY, Kim JH, Choi JH, Park S, Hwang YI, Jang SH, et al. The basophil activation test is safe and useful for confirming drug-induced anaphylaxis. Allergy Asthma Immunol Res. 2016;8(6):541-544. doi: 10.4168/aair.2016.8.6.541.
20. MacGlashan D. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. Clin Exp Allergy. 2010;40(9):1365-77. doi: 10.1111/j.1365-2222.2010.03572.x.
21. Mayorga C, Ebo DG, Lang DM, Pichler WJ, Sabato V, Park MA, et al. Controversies in drug allergy: In vitro testing. J Allergy Clin Immunol. 2019;143(1):56-65. doi: 10.1016/j.jaci.2018.09.022.
22. Koumaki D, Gregoriou S, Evangelou G, Katoulis A, Papadakis M, Krueger-Krasagakis SE, et al. Real-life utility of basophil activation test in the diagnosis of immediate hypersensitivity drug reactions. Dermatol Ther (Heidelb). 2023;13(12):3229-3239. doi: 10.1007/s13555-023-01069-w.
23. Mayorga C, Ariza A, Muñoz-Cano R, Sabato V, Doña I, Torres MJ. Biomarkers of immediate drug hypersensitivity. Allergy. 2024;79(3):601-612. doi: 10.1111/all.15933.
24. Worm M, Pazur K, Morakabati P, Redhu D. IgE and non-IgE-mediated pathways in anaphylaxis. Semin Immunopathol. 2025;47(1):34. doi: 10.1007/s00281-025-01056-7.
25. Campos L, Giavina-Bianchi P, Acharya S, Lynch DM, Kalil J, Castells M. Basophil Activation Test as a Biomarker for Taxanes Anaphylaxis. Front Allergy. 2022;3(787749):1-9. doi: 10.3389/falgy.2022.787749.
26. Pałgan K. Mast Cells and Basophils in IgE-Independent Anaphylaxis. Int J Mol Sci. 2023;24(16):1-15, 2023. doi: 10.3390/ijms241612802.
27. Syrigou E, Makrilia N, Koti I, Saif MW, Syrigos KN. Hypersensitivity reactions to antineoplastic agents: an overview. Anticancer Drugs. 2009;20(1):1-6. doi: 10.1097/CAD.0b013e32831961b3.
28. Doña I, Bogas G, Salas M, Testera A, Moreno E, Laguna JJ, et al. Hypersensitivity Reactions to Multiple Iodinated Contrast Media. Front Pharmacol. 2020;11:575437. doi: 10.3389/fphar.2020.575437 2020.
29. Kühl J, Bergh B, Laudes M, Szymczak S, Heine G. Cofactors of drug hypersensitivity—A monocenter retrospective analysis. Front Allergy. 2023;3:1097977. doi: 10.3389/falgy.2022.1097977.
30. Pagani M, Bavbek S, Alvarez-Cuesta E, Berna Dursun A, Bonadonna P, Castells M, et al. Hypersensitivity reactions to chemotherapy: an EAACI Position Paper. Allergy. 2022;77(2):388-403. doi: 10.1111/all.15113.
31. Nunes FA, Brandao LS, Guerzet Ayres Bastos P, Guerzet P, Lacerda AE, Piza CSFT, et al. Drug Hypersensitivity Reactions in Children: Are there factors associated with severity? J Allergy Clin Immunol. 2019;143(2):AB161.
32. Doña I, Torres MJ, Celik G, Phillips E, Tanno LK, Castells M. Changing patterns in the epidemiology of drug allergy. Allergy. 2024;79(3):613-628. doi: 10.1111/all.15970.
33. Salas M, Fernández-Santamaría R, Mayorga C, Barrionuevo E, Ariza A, Posadas T, et al. Use of the Basophil Activation Test May Reduce the Need for Drug Provocation in Amoxicillin-Clavulanic Allergy. J Allergy Clin Immunol Pract. 2018;6(3):1010-1018.e2. doi: 10.1016/j.jaip.2017.08.009.
34. Felix MMR, Kuschnir FC, Boechat JL, Castells M. Recent findings on drug hypersensitivity in children. Front. Allergy. 2024;5:1330517. doi: 10.3389/falgy.2024.1330517.
35. Nakamura N, Tamagawa-Mineoka R, Masuda K, Katoh N. Immediate-type allergic reactions to local anesthetics. Allergology International. 2018;67(1):160-161. doi: 10.1016/j.alit.2017.07.003.
36. Ramírez CE, Domingo MV, Font AP, Esteller PG, Marchuet MJC, Foix MPS, et al. Do cross-food allergies to propofol exist? Anesth Pain Med (Seoul). 2022;17(4):381-385. doi: 10.17085/apm.22195.
37. Zuo J, Gong R, Liu X, Zhao J. Risk of True Allergy to Local Anesthetics: 10-Year Experience from an Anesthesia Allergy Clinic in China. Ther Clin Risk Manag. 2020;16:1297-1303. doi: 10.2147/TCRM.S280259.
38. Schindewolf M, Recke A, Zillikens D, Lindhoff-Last E, Ludwig RJ. Nadroparin carries a potentially high risk of inducing cutaneous delayed-type hypersensitivity responses. Contact Dermatitis. 2017;77(1):35-41. doi: 10.1111/cod.12764.
39. Bai W, Zhang X, Sun S, Wang O, Li C, Zhang X, et al. Effect of low-molecular-weight heparins on anti-Xa peak levels and adverse reaction in Chinese patients with recurrent spontaneous abortion: a single-center, observational study. BMC Pregnancy Childbirth. 2021;21:683. doi: 10.1186/s12884-021-04161-1.
40. Powell AK, Yates EA, Fernig DG, Turnbull JE. Interactions of heparin/heparan sulfate with proteins: Appraisal of structural factors and experimental approaches. Glycobiology. 2004;14(4):17R-30R. doi: 10.1093/glycob/cwh051.
41. Kashiwakura Y, Kojima H, Kanno Y, Hashiguchi M, Kobata T. Heparin affects the induction of regulatory T cells independent of anti-coagulant activity and suppresses allogeneic immune responses. Clin Exp Immunol. 2020;202(1):119-135. doi: 10.1111/cei.13480.
42. Das M, Stephen-Victor E, Bayry J. Regulatory T cells do not suppress rather activate human basophils by IL-3 and STAT5-dependent mechanisms. OncoImmunology. 2020;9(1). doi: 10.1080/2162402X.2020.1773193.
43. Ipinmoroti AO, Pandit R, Crenshaw BJ, Sims B, Matthews QL. Human adenovirus type 3 restores pharmacologically inhibited exosomal cargo in lung carcinoma cells. Front Pharmacol. 2024;15:1339862. doi: 10.3389/fphar.2024.1339862.
44. Chen T, Lan L, Sun W, Yu J, Lu B, Chen L, et al. Localized and Generalized Skin Adverse Drug Reactions to Nadroparin Calcium Injection in 6 Cases of Pregnant Women. J Healthc Eng. 2022;2022:5622482. doi: 10.1155/2022/5622482.
45. Xu X, Dai Y. Heparin: an intervenor in cell communication. J Cell Mol Med. 2010;14(1-2):175-80. doi: 10.1111/j.1582-4934.2009.00871.x.

46. Bircher AJ, Harr T, Hohenstein L, Tsakiris DA. Hypersensitivity reactions to anticoagulant drugs: diagnosis and management options. Allergy. 2006;61(12):1432-1440. doi: 10.1111/j.1398-9995.2006.01227.x.
47. Schultinge L, Knol HM, Kluin-Nelemans HC, Erwich JJHM, Meijer K. Incidence of hypersensitivity skin reactions in patients on full-dose low-molecular-weight heparins during pregnancy. Neth J Med. 2013;71(10):518-22.
48. Ebo DG, Haine SE, Hagendorens MM, Bridts CH, Conraads VM, Vorlat A, et al. Hypersensitivity to Nadroparin Calcium. Clin Drug Invest. 2004;24(7):421-426. doi: 10.2165/00044011-200424070-00005.
49. Ali S, Udrea R, Boustani R, Puiu IA, Corcea SL, Spiru L. Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs: does age matter? Arch Clin Cases. 2022;9(2):80-88. doi: 10.22551/2022.35.0902.10208.
50. Blanca-Lopez N, Agundez JAC, Fernández J, García-Martin E, Blanca M. Ibuprofen and Other Arylpropionics: The Relevance in Immediate Hypersensitivity Drug Reactions. Int Arch Allergy Immunol. 2024;185(10):953-963. doi: 10.1159/000539043.
51. Rodríguez-Trabado A, Cámara-Hijón C, Ramos-Cantariño A, Porcel-Carreño SL, Jiménez-Timón S, Pereira-Navarro G, et al. Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. Allergy Asthma Proc. 2008;29(3):241-9. doi: 10.2500/aap.2008.29.3114.
52. Abuaf N, Rostane H, Barbara J, Toly-Ndour C, Gaouar H, Mathelier-Fusade P, et al. Comparison of CD63 Upregulation Induced by NSAIDs on Basophils and Monocytes in Patients with NSAID Hypersensitivity. J Allergy. 2012;2012;580873. doi: 10.1155/2012/580873.
53. Blanca-López N, Pérez-Sánchez N, Agúndez JA, García-Martin E, Torres MJ, Cornejo-García JA, et al. Allergic Reactions to Metamizole: Immediate and Delayed Responses. Int Arch Allergy Immunol. 2016;169(4):223-30. doi: 10.1159/000444798.
54. Trautmann A, Brockow K, Stoevesandt J. Metamizole-induced reactions as a paradigm of drug hypersensitivity: Non-allergic reactions, anaphylaxis, and delayed-type allergy. Clin Exp Allergy. 2020;50(9):1103-1106. doi: 10.1111/cea.13689.
55. Requena GM. Basophil Activation Test by Flow Cytometry in Patients with Severe Immediate Allergic Reactions To Pyrazolones. Allergy Clin Immunol. 2008;121(2):Suppl 1:S190.
56. Teles A, Ribeiro-Mourão F, Branco M, Araújo AR, Vieira T. Paracetamol Allergy: A Case of a 9-Year-Old Female with a History of Atopy. Pediatr Allergy Immunol Pulmonol. 2021;34(2):80-82. doi: 10.1089/ped.2021.0027.
57. Limão R, Spínola Santos A, Conceição Pereira Santos M, Pedro E, Lopes A. An Unusual IgE-Mediated Hypersensitivity: Two Case Reports of Paracetamol Allergy. Cureus. 2023;15(7):1-4, e42240. doi: 10.7759/cureus.42240.
58. Bergeret A, Bérard F, Nicolas JF, Hacard F, Nosbaum A, Jaulent C. Detection of an immediate hypersensitivity to paracetamol after drug challenge. World Allergy Organ J. 2020;13(8):83-84, P144.
59. Accarino JJO, Ramsey A, Samarakoon U, Phillips E, Gonzalez-Estrada A, Otani IM, et al. Drug allergy in older adults: A study from the United States Drug Allergy Registry. Ann Allergy Asthma Immunol. 2023;131(5):628-636.e2. doi: 10.1016/j.anai.2023.07.024.
60. Sun D, Fernandes G. Lovastatin inhibits BM-derived dendritic cell maturation and upregulates proinflammatory cytokine production. Cell Immunol. 2003;223(1):52-62. doi: 10.1016/S0008-8749(03)00148-5.
61. Chamani S, Kooshkaki O, Moossavi M, Rastegar M, Soflaei SS, McCloskey AP, et al. The effects of statins on the function and differentiation of blood cells. Arch Med Sci. 2023;19(5):1314-1326. doi: 10.5114/aoms/158546.
62. Kolawole EM, McLeod JJ, Ndaw V, et Abebayehu D, Barnstein BO, Faber T, et al. Fluvastatin suppresses mast cell and basophil IgE responses: genotype-dependent effects. J Immunol. 2016;196:1461-70. doi: 10.4049/jimmunol.1501932.
63. Kulshreshtha A, Singh S, Ahmad M, Khanna K, Ahmad T, Agrawal1 A, et al. Simvastatin mediates inhibition of exosome synthesis, localization and secretion via multicomponent interventions. Sci Rep. 2019;9:16373. doi: 10.1038/s41598-019-52765-7.
64. Chmielewski PP, Data K, Strzelec B, Farzaneh M, Anbiyaiee A, Zaheer U, et al. Human Aging and Age-Related Diseases: From Underlying Mechanisms to Pro-Longevity Interventions. Aging Dis. 2024 Jun 2;16(4):1853-1877. doi: 10.14336/AD.2024.0280.
65. Sun H, Xia T, Ma S, Lv T, Li Y. Intercellular communication is crucial in the regulation of healthy aging via exosomes. Pharmacol Res. 2025;212:107591. doi: 10.1016/j.phrs.2025.107591.
66. Beinert T, Munzing S, Possinger K, Krombach F. J. Increased expression of the tetraspanins CD53 and CD63 on apoptotic human neutrophils. Leukoc. Biol. 2000;67(3):369-373. doi: 10.1002/jlb.67.3.369.
67. Lu B, Chen HD, Lu HG. The relationship between apoptosis and aging. Adv Biosci Biotechnol. 2012;3:705-711. doi:10.4236/abb.2012.326091.
68. O'Grady S, Crown J, Duffy MJ. Statins inhibit proliferation and induce apoptosis in triple-negative breast cancer cells. Med Oncol. 2022;39(10):142. doi: 10.1007/s12032-022-01733-9.
69. Caffario P, Dammacco F, Gernone A, Silvestris F. Statins activate the mitochondrial pathway of apoptosis in human lymphoblasts and myeloma cells. Carcinogenesis. 2005;26(5):883-891. doi: 10.1093/carcin/bgi036.
70. Ventura MT, Boni E, Cecere R, Buquicchio R, Calogiuri GF, Martignago I, et al. Importance of hypersensitivity in adverse reactions to drugs in the elderly. Clin Mol Allergy. 2018;16(7):1-8. doi: 10.1186/s12948-018-0083-x.
71. Bielen C, Bielen L, Liki R. Incidence, etiology, predictors and outcomes of suspected drug hypersensitivity reactions in a tertiary care university hospital's emergency department: A retrospective study. Wien Klin Wochenschr. 2019;131(13-14):329-336. doi: 10.1007/s00508-019-1499-0.
72. Sella JA, Ferriani MPL, Melo JML, Neto OT, Zanetti MET, Cordeiro DL, et al. Type I and type IIb autoimmune chronic spontaneous urticaria: Using common clinical tools for endotyping patients with CSU. J Allergy Clin Immunol Global. 2023;2:100159. doi: 10.1016/j.jacig.2023.100159.
73. Asero R. Nonsteroidal anti-inflammatory drugs hypersensitivity in chronic spontaneous urticaria in the light of its pathogenesis. Eur Ann Allergy Clin Immunol. 2022;54(4):189-191. doi: 10.23822/EurAnnACI.1764-1489.227.
74. Netchiporouk E, Moreau L, Rahme E, Maurer M, Leitenyi D, Ben-Shoshan M. Positive CD63 Basophil Activation Tests Are Common in Children with Chronic Spontaneous Urticaria and Linked to High Disease Activity. Int Arch Allergy Immunol. 2016;171(2):81-88. doi: 10.1159/000451084.
75. Pallardy M, Bechara R, Whritenour J, Mitchell-Ryan S, Herzyk D, Lebrec H, et al. Drug hypersensitivity reactions: review of the state of the science for prediction and diagnosis. Toxicol Sci. 2024;200(1):11-30. doi: 10.1093/toxsci/kfae046.

EDRA ORIGINAL ARTICLE

Eur Ann Allergy Clin Immunol Vol 58, N.6, 263-268, 2026

David Longhino, Caterina Sarnari, Gloria Andriollo, Arianna Aruanno, Angela Rizzi, Eleonora Nucera, Alessandro Buonomo

Allergy evaluation in hypersensitivity to platinum compounds and taxanes: a 5-year experience

Allergy and Clinical Immunology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

Key words

Hypersensitivity; platinum compounds; taxanes; skin test; cross-reactivity.

Corresponding author
Arianna Aruanno
Allergy and Clinical Immunology Unit
Fondazione Policlinico Universitario A. Gemelli IRCCS
Università Cattolica del Sacro Cuore
largo Agostino Gemelli 8
00168 Rome, Italy
ORCID: 0000-0002-6746-9044
E-mail: arianna.aruanno@policlinicogemelli.it

Doi
10.23822/EurAnnACI.1764-1489.389

Impact statement

In this paper, we report our comprehensive experience of allergological evaluation by skin tests in 276 patients who experienced a hypersensitivity reaction to platinum compounds and/or taxanes.

Summary

Background. *Platinum compounds (PC) and taxanes (TX) are drugs frequently administered for the treatment of several cancers. In recent years, their increased use has been accompanied by a relatively high prevalence of hypersensitivity reactions (HSRs). In this study, we report our comprehensive experience of allergy skin test (ST) evaluation in 276 patients who experienced HSRs to PC and/or TX.*

Primary objectives were to determine the clinical characteristics of HSRs, and to assess the role of STs in the diagnosis and prevention of HSRs in order to address the subsequent treatments. Secondary objective was to evaluate any cross-reactivity between PC (cisplatin, oxaliplatin and carboplatin) and among TX (paclitaxel and docetaxel). ***Methods.*** *A monocentric, retrospective, observational, cohort study including patients who experienced one or more HSR during PC and/or TX chemotherapy was conducted from May 2018 to July 2023 in the Allergy and Clinical Immunology Unit at the "Fondazione Policlinico Universitario A. Gemelli" IRCCS, Rome.* ***Results.*** *A total of 114 patients with positive ST were identified: 102 for PC, 11 for TX and one for both. 162 patients were tested negative. Patients with HSR to PC were found to be allergic in 64%, compared to 9.9% of patients with HSR to TX ($p < 0.0001$). Patients who experience more serious reactions have a higher chance to have a positive result on STs ($p < 0.01$).* ***Conclusions.*** *STs to investigate sensitization to TX or PC is a highly valuable diagnostic tool in selecting the appropriate therapy when a hypersensitivity reaction occurs.*

Introduction

Platinum compounds (PC) and taxanes (TX) are drugs frequently administered for the treatment of several cancers. In recent years, their increased use has been accompanied by a relatively high prevalence of hypersensitivity reactions (HSRs). When such an event occurs, the clinician is faced with the dilemma of choosing whether to continue the treatment, exposing the patient to the risk of further reactions, or whether to interrupt it and replace it with alternative drugs. Three PC are most frequently used in oncological patients: carboplatin, cisplatin and oxaliplatin. They can cause heterogeneous reactions (from mild skin reactions to severe cardiovascular anaphylaxis) and are usually observed after multiple administrations. Pathogenesis can result from type I (IgE- and non-IgE-mediated mechanisms), cytokine release and mixed reactions. The incidence of PC-HSRs ranges from 12% to 17%: carboplatin is the main culprit with an incidence up to 46% in patients who received seven administrations, while oxaliplatin can induce HSRs in about 10-19%, and cisplatin in about 5-20% (1, 2).

TX are antineoplastic agents used for the treatment of several cancers; they include paclitaxel, docetaxel, nab-paclitaxel, and cabazitaxel. In most patients, cutaneous signs (most commonly flushing) and back pain occur. Cardiovascular, respiratory and gas-

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

trointestinal symptoms may also happen. Pathogenesis of these reactions can result from type I IgE-mediated mechanism, direct activation of basophils, and IgG-mediated complement activation. Solvents for TX, Cremophor EL for paclitaxel and polysorbate 80 for docetaxel, can activate mast-cells by an IgE-mediated mechanism or through direct complement activation. The reported incidence of immediate TX-HSRs is between 10% to 70%, typically at first or second exposure (3-5).

Skin prick tests (SPT) and intradermal tests (IDT) are the main diagnostic tools for the identification of PC- and TX-HSRs. Skin tests (STs) concentrations and procedures are standardized and defined in accordance with the ENDA/EAACI recommendations (6).

According to guidelines (7, 8), allergy testing should be performed between 6 weeks and 6 months following the reaction. STs reported sensitivity is 66% for cisplatin and 100% for carboplatin and oxaliplatin, with a negative predictive value of 92% for PC and 100% for TX (2). Several cases of cross-reactivity between molecules of the same family were also highlighted, especially to cisplatin and oxaliplatin in patients who reacted to carboplatin, while less cross-reactivity has been highlighted between cisplatin and other PC (9, 10).

Regarding the diagnostic role of patch testing, it is not recommended; the study of delayed reactions can be carried out through delayed reading (24 hours, 48, up to 7 days) of intradermal reactions (11).

In this study, we report our comprehensive experience of allergy STs evaluation in 276 patients who experienced HSRs to PC and/or TX.

Materials and methods

Study design and objectives

A monocentric, retrospective, observational, cohort study including patients who experienced one or more HSR during PC and/or TX chemotherapy was conducted from May 2018 to July 2023 and was approved by the local ethical committee ("Comitato Etico Territoriale Lazio Area 3 - approval number: 3883). STs were conducted in the Allergy and Clinical Immunology Unit at the "Fondazione Policlinico Universitario A. Gemelli" IRCCS, Rome.

Primary objectives were:

a) to determine the clinical characteristics of HSRs;
b) to assess the role of STs in the diagnosis and prevention of HSRs in order to address the subsequent treatments.

Secondary objective was to evaluate any cross-reactivity between PC (cisplatin, oxaliplatin and carboplatin) and among TX (paclitaxel and docetaxel).

Materials and allergy evaluation

The patients were identified based on clinical symptoms that were compatible with immediate HSRs according to the criteria set by the ENDA/EAACI Interest Group on Drug Hypersensitivity (6). Clinical data were collected, and the HSRs were reviewed and classified by an Allergy specialist according to Brown's classification (12).

Inclusion criteria were age ≥ 18 years and a clinical history of immediate HSR after the administration of PC (carboplatin, oxaliplatin or cisplatin) or TX (paclitaxel or docetaxel).

Exclusion criteria were instead a clinical history of delayed HSR and a diagnosis of previous Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), acute generalized exanthematous pustulosis (AGEP), Drug Rash with Eosinophilia and Systemic Symptoms (DRESS).

An allergist gathered a detailed history of the patients' HSR, and STs were performed at least 3 weeks after the reaction. Histamine and saline solutions were used as positive and negative controls, respectively. Both procedures were performed on the volar surface of the forearm. Firstly, SPTs were performed and, if negative, IDTs were carried out till the maximum non-irritant concentration (**table I**). STs were read according to EAACI guidelines.

Table I *- STs Concentration for PC and TX.*

	SPT concentration	IDT concentration
Carboplatin	10 mg/ml	1 mg/ml
		5 mg/ml
		10 mg/ml
Cisplatin	1 mg/ml	0.01 mg/ml
		0.1 mg/ml
		1 mg/ml
Oxaliplatin	5 mg/ml	0.05 mg/ml
		0.5 mg/ml
		5 mg/ml
Paclitaxel	6 mg/ml	0.1 mg/ml
		1 mg/ml
Docetaxel	10 mg/ml	0.1 mg/ml
		1 mg/ml

Sample size

For calculation of the sample size, we considered: 1) a population of 276 patients with a HSR to PS and/or TX; 2) a margin error of 10%; and 3) a chosen sampling confidence level of 90%.

Statistical analysis

The sample was described in its clinical and demographic characteristics through descriptive statistics techniques. The qualitative variables are represented by tables of absolute frequencies

and percentages. Qualitative variables have been summarized with mean and standard deviations. The normality of data has been verified with the Kolmogorov-Smirnov test, and the proportions were compared applying the Chi-square test. A P-value of less than 0.05 was considered statistically significant. All the statistical analyses have been performed with Statistical Package for the Social Sciences (SPSS) statistical software (Released 2017, IBM SPSS Statistics for Windows, Version 25.0; IBM Corp., Armonk, NY).

Results

Patient characteristics

Over 5 years, 276 female patients with a clinical history of at least one HSR to PC and/or TX were enrolled. 155 patients presented a reaction with PC, 115 with TX, 6 patients with both drugs. Patients' ages ranged from 27 to 87 years (mean, 59 years). The most common malignancies were ovarian (69.6%), endometrial (10.5%) and cervical (9%), follow by mammal and rectum. 105 patients were treated for primary cancers, 166 were treated for recurrent cancers, and for 5 patients no data were available. The most common agent responsible for hypersensitivity was carboplatin in 146 (51.8%) patients, followed by paclitaxel in 121 (42.9%), cisplatin 11 (3.9%), and oxaliplatin 4 (1.4%).

HSRs characteristics

224 patients had only one reaction (81.1%), 43 patients had two (15.6%), 8 patients had three (2.9%) and only one patient had four. A mild reaction was experienced by 32.6% of patients, 43.8% of patients presented a moderate reaction and 23.6% experienced a severe reaction.

The most common symptoms were cutaneous, respiratory, gastrointestinal, neurological, and cardiovascular.

Platinum compounds HSRs

A total of 161 patients had reactions with at least one platinum compound: 146 with carboplatin, 11 with cisplatin and 4 with oxaliplatin.

The HSRs to PC were recorded mainly during the II-III cycle of therapy, but it is important to underline that most of the reactions occurred during the second therapeutic line, therefore identifying the eighth or ninth administration of the drug. The main symptoms were cutaneous (erythema) and respiratory (dyspnea). Of all the reactions, 29.8% were mild, 42.3% moderate and 27.9% were severe.

Taxanes HSRs

All 121 patients reacted after the exposure to paclitaxel, and the majority (92.5%) reacted to the first or second exposure despite the use of premedication. 80% of the patients were at their first therapy line.

The main symptoms were cutaneous (erythema), respiratory (dyspnea) and neuromuscular (back pain). Of all the reactions, 36.4% were mild, 45.5% moderate and 18.1% were severe.

Taxanes and platinum compounds HSRs

Six patients had reactions with both types of chemotherapy. In all these patients, carboplatin and paclitaxel were involved, and the reactions occurred during separate infusion sessions.

Skin test results

All patients with HSRs to PC and TX underwent ST (SPT and IDT) with the culprit drug and with cross-reacting drugs. The time interval between the reaction and the allergological evaluation was conducted between 3 weeks following the reaction (in 94 patients) up to over a year (38 patients), as summarized in **table II**.

A total of 114 patients with positive ST were identified: 102 for PC, 11 for TX and one for both. 162 patients were tested negative. Patients with HSR to PC were found to be allergic in 64%, compared to 9.9% of patients with HSR to TX ($p < 0.0001$) (**table III**).

Table II *- Weeks between the HSR and the allergological exam.*

Time interval between ST and reaction	n patients (%)
3 weeks	94 (34.05%)
4 weeks	60 (21.8)
5 weeks	25 (9.05%)
6 weeks	11 (3.98%)
6 weeks - 6 months	38 (13.76%)
6 months - 1 year	10 (3.62%)
>1 year	38 (13.76%)

Table III *- Comparison between HSRs.*

		PC HSRs	TX HSRs	PC + TX HRSs	P-value
STs	Positive	102	11	1	
	Negative	53	104	5	
Total		**155**	**115**	**6**	**< 0.0001**

Skin test for platinum agents

Skin reactions were positive for at least one platinum salt in 103/161 patients (64%), while 58/161 patients (36%) had negative STs. 76 patients tested positive for only 1 agent, 22 patients tested positive for 2 platinum agents, while 5 patients tested positive for all 3 molecules.

In total, 22 patients had positive ST for cisplatin, 100 for carboplatin and 13 for oxaliplatin. Of the 22 cisplatin-positive patients, only 6 had a history of HSR with this drug: of the remaining, 15 had reacted to carboplatin and 1 to oxaliplatin. Of the 100 patients who presented a positive allergy test for carboplatin, this was implicated in 99 reactions, while in only one patient the culprit drug was oxaliplatin. 13 patients had positive STs for oxaliplatin, but only in 2 occasions this drug was responsible for the HSR. Over 48 patients with grade 1 reaction, 23 had positive ST (47.9%), while over the 68 patients with moderate (grade 2) reaction we found 44 positivity (64.7%), and among 45 patients with grade 3 reactions, 37 were positive (82.2%), meaning that patients with more severe reactions have a higher possibility of being positive compared to patients with minor reactions ($p < 0.01$) (**table IV**).

Table IV *- ST positivity according to the type of reaction in patients treated with PC.*

Grade of reaction according to Brown's classification (n patients)	STs positivity, n (%)
I (48)	23 (47.9%)
II (68)	44 (64.7%)
III (45)	37 (82.2%)

Skin test to taxanes

STs were positive for at least one taxane agent in 12 patients over 121 (9.9%), while were negative in 109 patients. All patients reacted to paclitaxel and all of them presented a positive ST to this agent. In 9/12 patients (75%), the allergological exam tested positive also for docetaxel. Over 44 patients with grade 1 reaction, only 1 had positive ST (2.3%), while over the 55 patients with moderate (grade 2) reaction we found 2 positivity (3.6%). Indeed among 22 patients with severe HSRs, ST were positive in 9 (40.9%), meaning that patients who experience more serious reactions have a higher chance to have a positive result on ST (P-value < 0.01) (**table V**).

Table V *- ST positivity according to the type of reaction in patients treated with TX.*

Grade of reaction according to Brown's classification (n patients)	STs positivity, n (%)
I (44)	1 (2.3%)
II (55)	2 (3.6%)
III (22)	9 (40.9%)

Discussion and conclusions

HSRs to PC occurred mainly in patients already chemo-treated (91% of patients) during the II-III cycle of therapy, which in the re-treated patients corresponds to the VIII-IX cycle. These data are in agreement with studies in the literature which highlight how the risk of developing an adverse reaction to PC increases with the progressive number of exposures; in detail, for cisplatin and carboplatin a significant increase in the risk of reaction is described after a median of 8 cycles, particularly during re-treatment for relapse, with a risk of HSR up to 100% at the third treatment (1). Furthermore, in accordance with literature data (13), reactions of moderate severity (grade 2 according to Brown's classification) mainly occurred (approximately 42.2%) and the most common manifestations were skin symptoms and dyspnea. Although the severity of the reaction is statistically correlated with the ST result, 47.9% of patients who had a grade I reaction to a PC were found to be allergic; this high percentage indicates that an allergological evaluation would also be indicated in these patients in order to avoid more severe reactions in subsequent infusions.

HSRs to TX occurred in almost all cases (92.5%) during administration of the first infusions (I-II) with moderate severity (45.5% grade 2 reactions); this timing of onset can be explained by the fact that the underlying pathogenetic mechanism is rarely immune-mediated, but often induced by complement activation or direct degranulation of mast cells and/or basophils.

In contrast, the high number of ST positivity in the PC-treated patients is indicative of a frequent underlying IgE-mediated mechanism; in fact, of 161 patients with HSRs to PC, 103 tested positive (64%), while of the 121 patients with HSRs to TX, only 12 (9.9%) tested positive (P-value < 0.0001). This result is comparable to those reported in the literature, in which a greater frequency of IgE-mediated reactions due to PC is found (14).

STs were performed within an average of five weeks from the time of the adverse reaction. Moreover, we found that 71 patients who tested positive underwent the allergological work-up before the 6 weeks defined by the guidelines and 40/71 already presented positive tests 3 weeks after the reaction. This could certainly accelerate the resumption of chemotherapy, but could also lead to false negative tests (8). Therefore, these patients, after receiving a new infusion of the same drug via a desensitization protocol, could be retested; in fact, some patients tend to become positive after re-exposure to the drug (15).

Patients who remain negative may undergo a challenge, if they are low grade in the risk stratification estimated by the allergist. This procedure could also be used in patients who are tested 6 months after the reaction, as it has now been demonstrated that STs tend to become negative over time with a significant decrease in sensitivity (16).

There is no unanimous consensus in the literature on the concentrations to be used for STs; in our Centre, we use the same concentrations as those proposed by Alvarez-Cuesta *et al*. (17). Pradelli *et al*. found an excellent negative predictive value of ST (92% for PC and 100% for TX) using, for PC, the same concentrations as ours (for both SPT and IDT) while, for TX, a more concentrated dilution for IDT (the same as the highest concentration used for SPT) (2).

Pagani *et al*. suggested instead a more diluted maximum concentration for IDT with carboplatin, more concentrated for SPT with cisplatin, the same concentration for oxaliplatin, more diluted for IDT with paclitaxel and more diluted for both IDT and SPT with docetaxel (14). We could not follow those concentrations because our study started before its publication, however our concentrations did not deviate too much from those utilized in the literature and, in our experience, minimize the risk of false positives and false negatives.

In this work, possible cross-reactivity between drugs belonging to the same family was also evaluated: a cross-reactivity between all three PC was highlighted, since a positive allergy test was recorded for oxaliplatin in 10 patients and for cisplatin in 15 patients with IgE-mediated allergy to carboplatin and negative history for the other two molecules. Furthermore, in a patient with a positive history of reaction to oxaliplatin, the skin reactions were also positive for cisplatin. 5 patients tested positive for all 3 molecules, 4 of them had experienced an HSR to carboplatin and one to oxaliplatin. As regards TX, the recorded cross-reactivity was 75% as out of 12 patients with IgE-mediated hypersensitivity to paclitaxel, 9 had positive STs also for docetaxel in the absence of adverse reactions to this molecule (**table VI**).

Finally, when an IgE-mediated reaction occurs, a rapid drug desensitization (RDD) protocol is indicated to allow patients who have no other treatment option to continue their therapy. Numerous cases of patients successfully treated with a 12-RDD protocol are described in the literature, but this is beyond the scope of this article and certainly deserves to be analyzed in future works (18). HSRs to chemotherapy are an increasingly widespread problem that requires correct management. STs to investigate sensitization to TX or PC in patients undergoing these therapies is a highly valuable diagnostic tool in selecting the appropriate therapy when a hypersensitivity reaction occurs, particularly for PC. With regard to TX, however, since STs were positive in only 9.9% of patients who experienced an HSR, it would be more appropriate to assess the severity of the reaction presented, despite the negativity of the STs, before selecting the subsequent therapy.

Table VI *- Cross-reactivity between PC and TX.*

Drug reaction (ST positivity with the culprit drug, n)	**Cross-reactivity in STs (ST positivity with other drugs, n)**		
	Carboplatin	**Oxaliplatin**	**Cisplatin**
Carboplatin (99)	/	1	0
Oxaliplatin (2)	10	/	1
Cisplatin (6)	15	1	/
	Cross-reactivity in STs (ST positivity with other drug, n)		
	Paclitaxel	**Docetaxel**	
Paclitaxel (12)	/	9	
Docetaxel (0)	/	/	

On regard of PC, 76 patients tested positive for only 1 agent, 22 patients tested positive for 2, while 5 patients tested positive for all 3 molecules.

Fundings

None.

Contributions

DL: data curation, formal analysis, project administration, software, writing – review & editing. CS, AB: conceptualization, data curation, investigation, methodology, project administration, writing – original draft. GA: conceptualization, data curation, investigation, methodology, project administration, writing – original draft. AA: supervision, validation, visualization, writing – review & editing. AR: supervision, validation, visualization, writing – review & editing. EN: supervision, validation.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Caiado J, Castells M. Presentation and Diagnosis of Hypersensitivity to Platinum Drugs. Curr Allergy Asthma Rep. 2015;15(4):15. doi: 10.1007/s11882-015-0515-3.
2. Pradelli J, Verdoire P, Boutros J, Frin AC, Follana P, Duquesne J, et al. Allergy Evaluation of Hypersensitivity to Platinum Salts and Taxanes: A Six-Year Experience. J Allergy Clin Immunol Pract. 2020;8(5):1658-1664. doi: 10.1016/j.jaip.2019.12.032.
3. Weiszhár Z, Czúcz J, Révész C, Rosivall L, Szebeni J, Rozsnyay Z. Complement activation by polyethoxylated pharmaceutical surfactants: Cremophor-EL, Tween-80 and Tween-20. Eur J Pharm Sci. 2012;45(4):492-8. doi: 10.1016/j.ejps.2011.09.016.
4. Picard M, Castells MC. Re-visiting Hypersensitivity Reactions to Taxanes: A Comprehensive Review. Clin Rev Allergy Immunol. 2015;49(2):177-91. doi: 10.1007/s12016-014-8416-0.
5. Picard M. Management of Hypersensitivity Reactions to Taxanes. Immunol Allergy Clin North Am. 2017;37(4):679-693. doi: 10.1016/j.iac.2017.07.004.
6. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, et al.; ENDA/EAACI Drug Allergy Interest Group. Skin test concentrations for systemically administered drugs -- an

ENDA/EAACI Drug Allergy Interest Group position paper. Allergy. 2013;68(6):702-12. doi: 10.1111/all.12142.
7. Pagani M, Bavbek S, Dursun AB, Bonadonna P, Caralli M, Cernadas J, et al. Role of Skin Tests in the Diagnosis of Immediate Hypersensitivity Reactions to Taxanes: Results of a Multicenter Study. J Allergy Clin Immunol Pract. 2019;7(3):990-997. doi: 10.1016/j.jaip.2018.09.018.
8. Romano A, Torres MJ, Castells M, Sanz ML, Blanca M. Diagnosis and management of drug hypersensitivity reactions. J Allergy Clin Immunol. 2011;127(3 Suppl):S67-73. doi: 10.1016/j.jaci.2010.11.047.
9. Meyer L, Zuberbier T, Worm M, Oettle H, Riess H. Hypersensitivity reactions to oxaliplatin: cross-reactivity to carboplatin and the introduction of a desensitization schedule. J Clin Oncol. 2002;20(4):1146-7. doi: 10.1200/JCO.2002.20.4.1146.
10. Pasteur J, Favier L, Pernot C, Guerriaud M, Bernigaud C, Lepage C, et al. Low Cross-Reactivity Between Cisplatin and Other Platinum Salts. J Allergy Clin Immunol Pract. 2019;7(6):1894-1900. doi: 10.1016/j.jaip.2019.01.057.
11. Leguy-Seguin V, Jolimoy G, Coudert B, Pernot C, Dalac S, Vabres P, et al. Diagnostic and predictive value of skin testing in platinum salt hypersensitivity. J Allergy Clin Immunol. 2007;119(3):726-30. doi: 10.1016/j.jaci.2006.11.640.
12. Brown SG. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol. 2004;114(2):371-6. doi: 10.1016/j.jaci.2004.04.029.
13. Caiado J, Picard M. Diagnostic tools for hypersensitivity to platinum drugs and taxanes: skin testing, specific IgE, and mast cell/basophil mediators. Curr Allergy Asthma Rep. 2014;14(8):451. doi: 10.1007/s11882-014-0451-7.
14. Pagani M, Bavbek S, Alvarez-Cuesta E, Berna Dursun A, Bonadonna P, Castells M, et al. Hypersensitivity reactions to chemotherapy: an EAACI Position Paper. Allergy. 2022;77(2):388-403. doi: 10.1111/all.15113.
15. Patil SU, Long AA, Ling M, Wilson MT, Hesterberg P, Wong JT, et al. A protocol for risk stratification of patients with carboplatin-induced hypersensitivity reactions. J Allergy Clin Immunol. 2012;129(2):443-7. doi: 10.1016/j.jaci.2011.10.010.
16. Li PH, Chiang V, Yeung HH, Au EY. Caution Against Temporary Tolerance and Negative Skin Testing During the Anergic Period Following Systemic Reactions. J Investig Allergol Clin Immunol. 2022;32(2):157-158. doi: 10.18176/jiaci.0724.
17. Alvarez-Cuesta E, Madrigal-Burgaleta R, Angel-Pereira D, Ureña-Tavera A, Zamora-Verduga M, Lopez-Gonzalez P, et al. Delving into cornerstones of hypersensitivity to antineoplastic and biological agents: value of diagnostic tools prior to desensitization. Allergy. 2015;70(7):784-94. doi: 10.1111/all.12620.
18. Castells MC, Tennant NM, Sloane DE, Hsu FI, Barrett NA, Hong DI, et al. Hypersensitivity reactions to chemotherapy: outcomes and safety of rapid desensitization in 413 cases. J Allergy Clin Immunol. 2008;122(3):574-80. doi: 10.1016/j.jaci.2008.02.044.

EDRA ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 58, N.6, 269-278, 2026

Catarina Novais[1], Ana Margarida Cruz[1,2], Ana Margarida Pereira[3,4,5], Rute Almeida[6], Rita Amaral[6,7,8], João Almeida Fonseca[5,6,9], Cristina Jácome[6]

Understanding beliefs about inhaled medication in patients with persistent asthma: a cross-sectional analysis of the INSPIRERS studies

[1]Bom Porto Family Health Unit, Santo António Local Health Unit, Porto, Portugal
[2]School of Medicine and Biomedical Sciences (ICBAS) of University of Porto, Porto, Portugal
[3]CINTESIS, Faculty of Medicine of University of Porto, Porto, Portugal
[4]Department of Community Medicine, Information and Health Decision Sciences (MEDCIDS), Faculty of Medicine, University of Porto, Porto, Portugal
[5]Allergy Unit, CUF Porto Hospital and Institute, Porto, Portugal
[6]CINTESIS@RISE, MEDCIDS, Faculty of Medicine of University of Porto, Porto, Portugal
[7]Deptartment of Cardiovascular and Respiratory Sciences, School of Health, Polytechnic Institute of Porto, Porto, Portugal
[8]Department of Women's and Children's Health, Paediatric Research, Uppsala University, Uppsala, Sweden
[9]MEDIDA – Medicina, Educação, Investigação, Desenvolvimento e Avaliação, Porto, Portugal

Key words

Asthma; beliefs; treatment adherence; illness perceptions; attitudes; real-world data.

Corresponding author
Cristina Jácome
Faculty of Medicine
University of Porto
Rua Dr. Plácido da Costa
4200-450 Porto, Portugal
ORCID: 0000-0002-1151-8791
E-mail: cristinajacome.ft@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.344

Impact statement

This study emphasizes the importance of patients' beliefs regarding asthma medications. Although the perception is mainly positive, comprehending patients' viewpoints enables targeted interventions that are crucial for enhancing patient outcomes.

Summary

Background. *Identifying factors influencing adherence, such as patients' beliefs about medication, is essential for effective asthma management. This study aims to assess and gain insight into the beliefs of patients with asthma regarding inhaled medication.* ***Methods.*** *This is a secondary analysis of the INSPIRERS studies. Patients aged ≥ 13 y.o., with persistent asthma and a prescription for inhaled controller were recruited from 60 primary and secondary care centers in Portugal from 2017 to 2020. Demographic and clinical characteristics were collected in a face-to-face visit. The Specific-Beliefs about Medicine Questionnaire was administered 1-week later by telephone interview. Mann-Whitney U and Kruskal-Wallis tests were used to explore relations between patients' beliefs and characteristics.* ***Results.*** *A total of 552 participants (mean 32.8 ± 17.3 y.o.; 64.5% female) were analyzed. The Necessity score (Median 19 [p25-p75 16,22]) was significantly higher than the Concerns score (15 [16,22], $p < 0.001$), resulting in a positive Necessity-Concern differential (Median 4 [0,7]). Acceptance (high necessity, low concerns) characterized 61% of participants, while 19% were ambivalent (high necessity, high concerns). Adolescents exhibited lower Necessity (Median 16 vs 20; $p < 0.001$) and Concerns scores (Median 11 vs 15; $p = 0.002$) than adults. In primary care setting, patients had significantly lower Necessity (Median 18 vs 19; $p = 0.027$) and Concerns (Median 14 vs 15; $p = 0.05$) compared to the secondary care.* ***Conclusions.*** *A predominantly positive perception of inhaled asthma medication necessity was found, although ambivalence or indifference exists in about 1/5 of patients. Our findings highlight the importance of personalized approaches to address beliefs and optimize patient education.*

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

Introduction

Asthma is a chronic respiratory disease that affects approximately 262 million people worldwide (1). It is a major noncommunicable disease that is associated with premature death and reduced quality of life, ranking 24th among the leading causes of years lived with disability. Asthma also represents a significant source of global economic burden, which can be reduced through strategies to improve access and adherence to evidence-based therapies (1, 2).

Adherence to inhaled controller medication is critical for effectively managing patients with asthma (3). However, adherence rates reported are highly variable, ranging from 2-63% (4, 5). Medication adherence has been described as particularly diminished in adolescents (6). Non-adherence to asthma therapy at any age leads to poor disease control, increasing the risk for asthma exacerbations, reducing quality of life, and raising healthcare utilization, resulting in high healthcare costs (4, 5, 7-10). Therefore, identifying and understanding the factors that contribute to poor adherence is essential for effective asthma management.

Adherence to long-term treatments for chronic diseases such as asthma is influenced by several factors that need to be considered when addressing adherence. These factors include not only social and economic aspects, the health care system, and the disease itself, but also patient-related characteristics. These include the patient's knowledge, expectations, perceptions, attitudes and beliefs (4). It is known that patients' beliefs and concerns about asthma and asthma medications are related to their medication-taking behavior and adherence to treatment (3-5, 11-13). Patients may have specific beliefs about the need for treatment to maintain the disease controlled, but also concerns about the adverse effects of adherence to such medications (14, 15). Patient's motivation to adhere to chronic medication is influenced by the balance between what they perceive to be a personal need or concern (15).

The Beliefs about Medicine Questionnaire-Specific (BMQ-Specific) is a commonly used patient-reported outcome measure to assess beliefs about medicines and has been shown to relate to non-adherence in patients with chronic illnesses, including asthma (16). Using the BMQ-Specific, previous studies have shown that patients' beliefs and concerns can be used to define different attitudinal groups associated with different levels of medication adherence (17, 18). Thus, understanding patients' beliefs about their inhaled medication is key to shared decision-making and to improving adherence. With this knowledge, healthcare professionals can intervene more effectively (12).

The existing literature describing the beliefs about medicines in patients with asthma is heterogeneous, often with small sample sizes and mostly recruited from a single setting (17-20). A recent study conducted in a large real-world adult population from the United States and five European countries concluded that, in line with previous studies, higher perceived necessity and lower concerns about medication were associated with better adherence in patients with asthma (21). It should be noted that Portugal was not included in this study and, to our knowledge, there is no real-world data available on the beliefs of Portuguese patients with asthma. While previous studies have explored the beliefs about medication in adults (14, 15, 17, 18, 21) and, to a lesser extent, in adolescents (19, 22), no studies have compared these beliefs in the two age groups.

The aim of this study is to assess and gain insight into the beliefs of adolescent and adult patients with asthma regarding inhaled medication.

Materials and methods

Study design

This study presents a secondary, cross-sectional analysis of four prospective multicenter observational studies conducted as part of the Inspirers project. The project aimed to improve adherence to asthma inhalers among patients with persistent asthma (23). Between November 2017 and October 2020, patients were recruited from 60 primary and secondary (allergy, pulmonology and pediatric) care centers in Portugal using convenience sampling. This study was conducted in accordance with the Declaration of Helsinki and corresponding ethical standards. The study protocol was approved by the ethics committees of all participating centers. Written informed consent was provided by all participants and/or legal representatives prior to enrolment in the study. Adolescents also signed a written informed assent. The study is reported according to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (24).

Population

Patients were included in the analysis if they had a previous medical diagnosis of persistent asthma, were at least 13 years old (13-17 years adolescents; ≥ 18 years adults) and had an active prescription for inhaled controller medication for asthma. All inhaled controller treatments were permitted, and no changes were made to any prescribed medication in relation to participation in these studies. Exclusion criteria included a diagnosis of a chronic lung disease other than asthma or any other significant chronic condition that could potentially interfere with the study's objectives.

Data collection

During the initial face-to-face visit, data were collected from both physicians and patients. Socio-demographic data (age, gender, education level, marital status, current occupation, civil parish, postal code) and clinical data (weight, height, smoking habits, age of asthma diagnosis, exacerbations) were collected from patients. Additionally, socioeconomic level was defined as the Portuguese Ecological Deprivation Index (EDI), extracted from the patient residence information (civil parish/postcode), and categorized

into 5 quintiles (Q1 = least deprived to Q5 = most deprived) (25). Information on the healthcare setting (primary, secondary) was obtained based on the center where the patients were recruited. Two questionnaires were used to assess asthma control: the Global Initiative for Asthma (GINA) assessment of symptoms control (3), completed by the physician, and the Control of Allergic Rhinitis and Asthma Test (CARAT) (26), completed by the patient. CARAT is a widely used self-administered questionnaire in clinical practice and research, adapted in over 27 languages (26). This questionnaire contains 10 questions, each rated on a 4-point Likert scale, with a total score ranging from 0 to 30 points. A score > 24 indicates good disease control (27). In addition, physicians also reported on the patients' current asthma treatment.

Approximately one week later, a telephone interview was conducted to administer the BMQ-Specific, the Morisky Medication Adherence Scale (MMAS-4) and a 1-100 numerical scale simulating a Visual analogue scale (VAS) of adherence to inhaled controller medication (28-30).

The study used the Portuguese version of the BMQ-Specific questionnaire to measure specific beliefs about inhaled medication (29). This patient-reported outcome measure had been previously applied by telephone (31). The BMQ-Specific consists of two subscales: Specific-Necessity and Specific-Concerns. The Necessity subscale contains five items and the Concerns subscale contains six items. Participants rated each item on a 5-point Likert scale, resulting in a total score ranging from 5 to 25 for the Necessity sub-scale and 6 to 30 in the Concerns subscale. A necessity-concerns differential was calculated by subtracting the Concerns subscale scores from the Necessity subscale scores, such that higher differential scores indicate higher perceived necessity and/or lower concerns, thereby representing lower likelihood of intentional non-adherence (29). Four attitudinal groups can be distinguished: skeptical (low necessity, high concerns), indifferent (low necessity, low concerns), ambivalent (high necessity, high concerns) and accepting (high necessity, low concerns) (17). Using Cronbach's alpha coefficient, the internal consistency of the total BMQ-Specific in this study was 0.723, while Cronbach's alphas for the Necessity and Concerns subscales were 0.779 and 0.719, respectively.

The MMAS-4 consists of four questions with yes (0) or no (1) response. The item scores are summed to determine three levels of adherence: 0 (high adherence), 1-2 (medium adherence), and 3-4 (low adherence) (32, 33). Additionally, patients reported their global adherence to asthma control inhalers during the previous week using a VAS, ranging from 0 (worst) to 100 (best). The VAS scores were grouped into three categories: low (0-50 mm), medium (51-80 mm) and high (81-100 mm). These categories are commonly used to differentiate adherence groups (30, 34, 35).

Statistical analysis

Descriptive statistics were used to characterize the sociodemographic and clinical characteristics of the sample and the BMQ-specific scores. Absolute and relative frequencies were used to characterize categorical variables. Means and standard deviation (SD) or medians and 25^{th}-75^{th} percentiles (p25-p75) were used, according to data distribution, to characterize the continuous variables. Normality of each variable was investigated with Kolmogorov-Smirnov tests and visual analysis of histograms. To explore relations between beliefs (necessity, concerns, necessity-concerns differential) and sociodemographic and clinical characteristics, Mann-Whitney U tests and Kruskal-Wallis tests were used. The level of significance considered was 0.05. Statistical analyses were performed using IBM SPSS Statistics V.29.0 (IBM Corporation, Armonk, New York, USA).

Results

Participants

A total of 589 participants were included in the study, but 37 did not answer the 1-week telephone interview and had no BMQ-Specific data. The analysis was conducted on 552 participants with asthma with a mean (±SD) age of 32.8 ± 17.3 (range 13-82) years old. Sixty-four percent (n = 356) of participants were women. About 41.7% (n = 230) of patients lived in deprived areas. Regarding healthcare setting, most patients (n = 426; 77.2%) were recruited in secondary care. According to the GINA assessment of symptom control, almost half of the participants had well-controlled asthma (n = 273; 49.5%) and about 51.4% (n = 284) had at least one exacerbation in the last year. **Table I** summarizes the socio-demographic and clinical characteristics of the participants.

Beliefs about inhaled medication

The median Necessity score (19 [16,22]) was significantly higher than the median Concerns score (15 [12,18], $p < 0.001$). The Necessity-Concerns differential was positive, with a median score of 4 [0,7]. Overall, patients had a high perception of the necessity of inhaled medication. Specifically, 94% of patients agreed that the inhaler protects them from getting worse, and 74% believed that their health depends on medication (**figure 1A**). However, almost half of the patients reported being worried about long term effects of medication (48%) and becoming too dependent on the inhaler (42%) (**figure 1B**). **Figure 1** shows the frequency of participants agreeing with the items of both BMQ subscales.

The patients were categorized in four groups for attitudinal analysis, as shown in **figure 2**. Most patients (61%; n = 338) demonstrated acceptance of their inhaled medication (high necessity, low concerns) and 19% (n = 261) exhibited ambivalence, reporting high necessity and high concerns regarding their inhaler. Only 3% of participants belonged to the skeptical group (low necessity, high concerns), while 17% of participants were indifferent towards their medication (low necessity, low concerns). In the

Table I *- Socio-demographic and clinical characteristics of the participants (n = 552).*

Characteristic	
Age (years) M ± SD[a]	32.8 ± 17.3
Age group n (%)	
Adolescent	153 (27.7)
Adult	399 (72.3)
Gender n (%)	
Female	356 (64.5)
Male	196 (35.5)
Educational level n (%)[b]	
No education completed	2 (0.4)
Primary school	36 (6.5)
Lower secondary education	191 (34.6)
Upper secondary education	161 (29.2)
Qualification above high school (but not university)	19 (3.4)
University or higher	134 (24.3)
Other	1 (0.2)
Marital status n (%)[c]	
Single	311 (56.3)
Married/Living as a couple	199 (36.1)
Separated/divorced	30 (5.4)
Widowed	11 (2)
Current occupation n (%)[d]	
Employed	259 (46.9)
Student	211 (38.2)
Unemployed	35 (6.3)
Retired	35 (6.3)
Other	8 (1.4)
Socioeconomic level[e]	
Q1-Q2 (least deprived)	154 (27.9)
Q3	105 (19.0)
Q4-5(most deprived)	230 (41.7)

Characteristic (Continued)	
BMI Kg/m^2, M ± SD[f]	24.8 (5.4)
Smoking Status n (%)[d]	
Never smoker	413 (74.8)
Ex-smoker	94 (17)
Current smoker	41 (7.4)
Setting n (%)	
Secondary care	426 (77.2)
Primary care	126 (22.8)
Age of asthma diagnosis (years) M ± SD[g]	16.5 ± 15.1
GINA assessment of symptom control n (%)[h]	
Well controlled	273 (49.5)
Partly controlled	173 (31.3)
Uncontrolled	101 (18.3)
CARAT n(%)	
Controlled	147 (26.6)
Uncontrolled	405 (73.4)
Exacerbations in the last year n (%)[i]	
None	253 (45.8)
At least one	284(51.4)
Inhaler prescribed n (%)[d]	
1	190 (34.4)
>1	358 (64.9)
MMAS-4 level n (%)[j]	
High	59 (10.7)
Medium	321 (58.2)
Low	172 (31.2)
VAS adherence level n(%)[k]	
High	241 (43.7)
Medium	150 (27.2)
Low	78 (14.1)

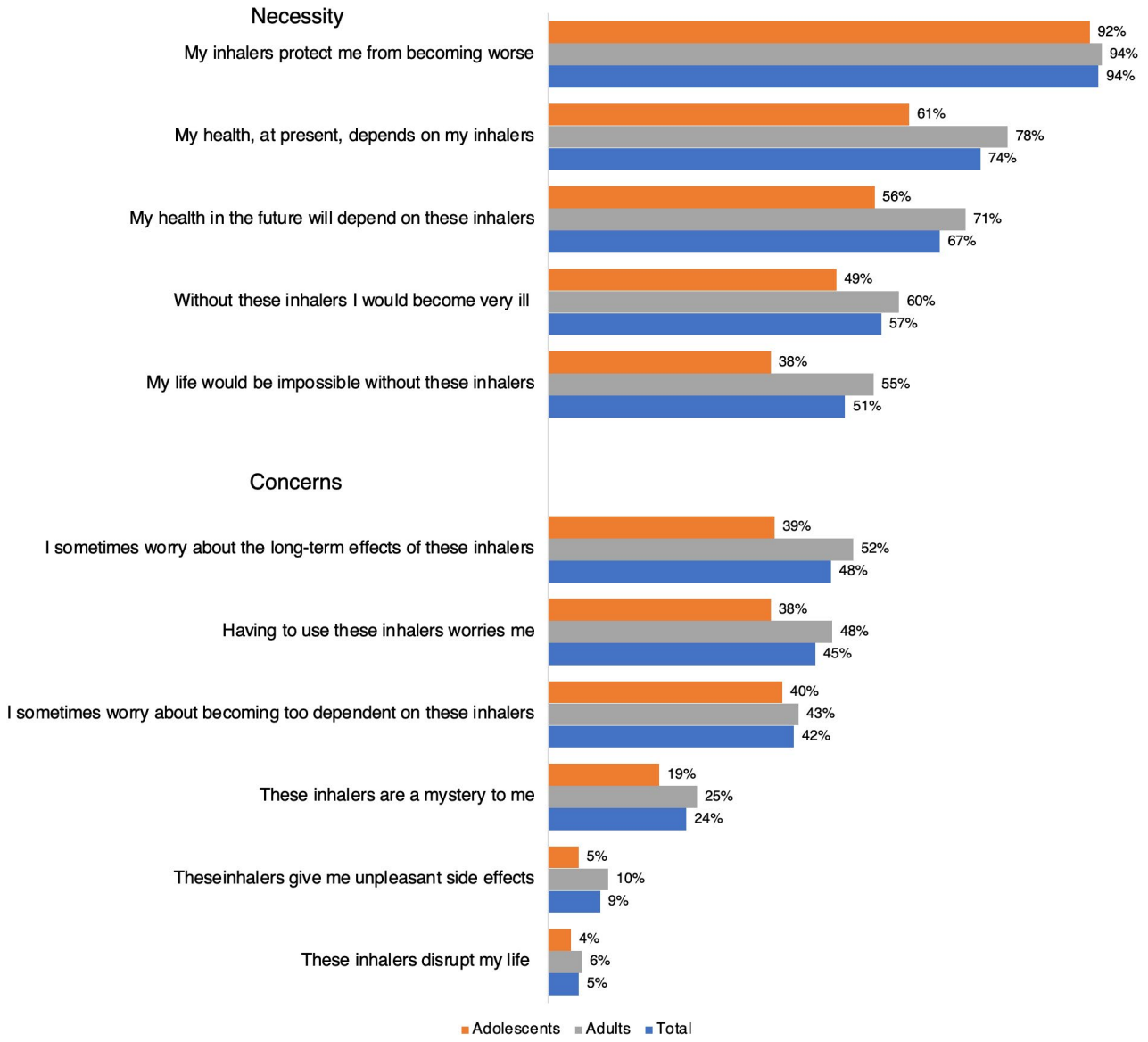
BMI: body mass index; CARAT-T: Control of Allergic Rhinitis and Asthma Test total score; GINA: Global Initiative for Asthma; M: Mean; MMAS-4: Morisky Medication Adherence Scale; SD: Standard Deviation; VAS: Visual analogue scale. [a]6 missing values. [b]7 missing values. [c] 1 missing value. [d]4 missing values. [e]63 missing values; [f]25 missing values. [g]18 missing values. [h]5 missing values. [i]15 missing values. [j]37 missing values. [k]83 missing values.

adolescents' group, the indifferent group was larger than the ambivalent group (22% *vs* 12%).

Beliefs and patients' characteristics

Table II summarizes the distribution of the Necessity, Concerns and Necessity-Concerns differential scores according to patient characteristics. Adolescents had significantly lower Necessity (Median [p25-75] 15.5 [18,20] *vs* 20 [17,22]; p < 0.001) and Concerns (Median 11 [14,17] *vs* 15 [12,18]; p = 0.002) scores compared to adults. Female patients had significantly higher Necessity scores (19 [16,22] *vs* 19[16,21]; p = 0.037) compared to male patients.

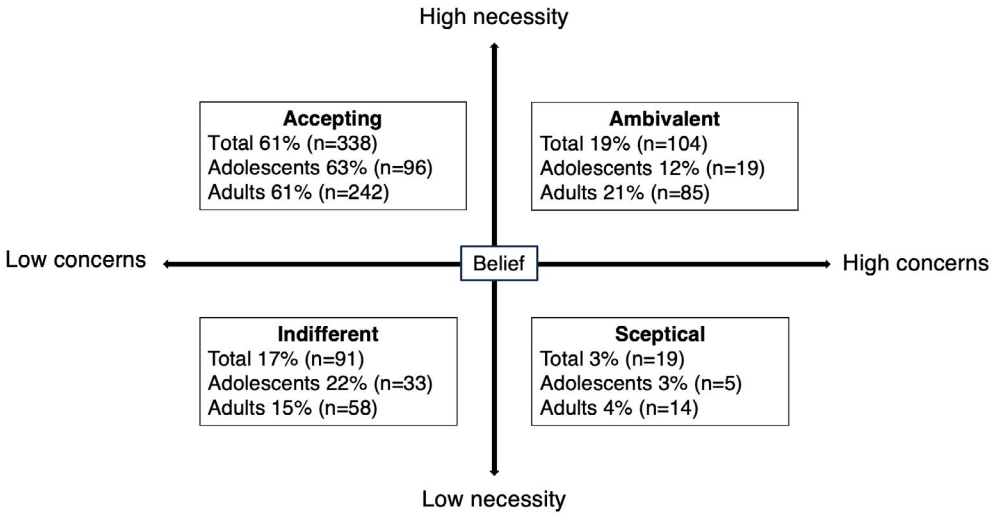
Figure 1 *- Frequency of patients agreeing with Necessity items and Concern items (n = 552).*



Regarding socioeconomic level, patients living in least-deprived regions (had significantly higher Necessity scores (20 [17,23] *vs* 19 [16,21]; p = 0.010) and Necessity-Concerns differential (5 [1,8] *vs* 3 [0,6]; p = 0.04) than those from most deprived regions. Patients recruited in primary care setting had significantly lower median scores for Necessity (18 [16,21] *vs* 19 [17,22]; p = 0.027) and Concerns (14 [11,17] *vs* 15 [12,18], p = 0.05) than those recruited in the secondary care setting.

In terms of disease control, the Necessity and Concerns scores were similar in participants with controlled and uncontrolled asthma, as determined by the GINA assessment of symptom control, CARAT or exacerbations in the last year. Patients prescribed with more than one inhaler had significant higher Necessity scores (Median 20 [17,22] *vs* 19 [16,21]; p = 0.007) compared to those prescribed with only one inhaler.

Figure 2 *- Frequency of patients with asthma in each attitudinal group based on their Necessity and Concerns scores (n = 552).*



When considering the MMAS-4, median Concerns scores were significantly different across the three adherence groups, being higher when adherence was lower. When considering VAS, the Necessity score of patients with higher adherence was significantly higher than patients with the lower adherence (Median 20 [17,22] *vs* 18 [14,21]; p = 0.008).

Discussion and conclusions

This study found that most patients with persistent asthma had a positive Necessity-Concern score differential, indicating that their need for medication outweighed their concerns. This highlights an overall positive perception of the need for inhaled medication among patients with asthma. On the other hand, almost half of the patients showed ambivalence towards their inhaled medication, with high levels of both necessity and concern.

Our findings are consistent with Van Steenis *et al.*, who reported that, on average, patients give a higher importance to the need for their asthma medication than to their concerns (18). Similar to previous studies (21, 36), the most reported concerns about inhaled medication were about their long-term effects, the general use of medication and becoming too dependent on their inhaler.

Looking at the attitudinal groups, the accepting group (61%), consisting of patients with high necessity and low concerns and the ambivalent group (19%), with both high necessity and high concerns, were the most represented. Accepting and ambivalent groups were also the most represented in a study including adults with asthma from 5 European countries (37) and both adolescents and adults with asthma from Spain and France (21).

It was previously described that patients in these two attitudinal groups, holding stronger necessity beliefs, exhibit higher adherence to medication (13, 17, 21). In our study, however, following the accepting group, the indifferent was the one with higher frequency in adolescents (22%). Previous studies have shown that the accepting group is predominant in adults (17, 18, 21), while the indifferent group predominates in adolescents (19, 22). As in previous articles, the proportion of patients in the skeptical group (low necessity, low concerns) was small (3%) (17, 18, 21).

When evaluating patients with asthma, it is essential to address their concerns and educate them to improve their understanding of medication, which can be achieved by demystifying fears and misconceptions (36). Personalized care strategies can help to improve medication-taking behavior and adherence to treatment. Policymakers and health systems together with community-based and patient and public involvement organizations can also play a role in improving asthma health literacy by developing and disseminating linguistically and culturally appropriate materials using different tools (written materials, videos, e-health tools) and outreach strategies (38).

In this study, the Necessity and Concerns scores were significantly lower in adolescents compared to adults. We did not find studies comparing beliefs among these two age groups, but the scores found in adolescents are consistent with findings in the literature (19, 22). This age group typically shows low levels of concern and necessity, despite taking the responsibility for taking their own medication. It is important to note that adolescence is a unique period of personal development, during which various factors may

Table II - *Beliefs (necessity, concerns, necessity-concern differential) and patient characteristics (n = 552).*

	Necessity	Concerns	Necessity-Concerns differential
Age group			
Adolescents	16 [8,20]*	11 [14,17]*	4 [0,7]
Adults	20 [17,22]	15 [12,18]	4 [0,8]
Gender			
Female	19 [16,22]*	15 [12,18]	4 [0,8]
Male	19 [16,21]	15 [12,18]	3 [0,7]
Socioeconomic level			
Q1-Q2 (least deprived)	20 [17,23]*	15 [12,18]	5 [1,8]*
Q3	19 [17,22]	14 [12,19]	4 [0,9]
Q4-5(most deprived)	19 [16,21]	15 [12,18]	3 [0,6]
Setting			
Secondary care	19 [17,22]	15 [12,18]	4 [0,7]
Primary care	18 [16,21]*	14 [11,17]*	4 [1,7]
GINA			
Well/Partly controlled	19 [16,22]	15 [12,17]	4 [0,8]
Uncontrolled	19 [16,22]	15 [12,19]	4 [0,7]
CARAT			
Controlled	19 [16,21]	14 [12,17]	5[0,8]
Uncontrolled	19 [16,22]	15 [12,19]	4 [0,7]
Exacerbations in the last year			
None	19 [16,22]	15 [12,18]	4 [0,7]
At least one	19 [16,22]	15 [12,18]	4 [0,7]
Inhaler prescribed			
1	19 [16,21]*	15 [12,18]	4 [0,7]
>1	20 [17,22]	15 [12,19]	4 [0,8]
MMAS-4			
High	19 [15,23]	14 [11,18]*	5 [2,7]
Medium	19 [17,22]	14 [12,18]	4 [0,8]
Low	19 [16,22]	16 [13,19]	3 [-1,6]
VAS adherence			
High	20 [17,22]*	15 [12,18]	4 [1,8]
Medium	19 [16,22]	15 [12,18]	4 [-1,7]
Low	18 [14,21]	16 [13,19]	2 [-3,6]

Data are presented as median [p25-p75]. CARAT-T: Control of Allergic Rhinitis and Asthma Test total score; GINA: Global Initiative for Asthma; MMAS-4: Morisky Medication Adherence Scale; VAS: Visual analogue scale. *p < 0.05

influence adherence and disease control (6). As a child grows into adolescence, autonomy and responsibility in taking asthma medication increases over time. By the age of 15 years old, approximately 75% of adolescents with asthma take responsibility for their controller medication (39). In this age group, adherence to asthma treatment may be influenced by factors such as social stigma or parental involvement, in addition to the patients' personal beliefs (40, 41). By showing that there are differences between adult and adolescent beliefs, this study highlights the need for personalized interventions and sheds light on the importance of understanding the unique factors that influence adherence in adolescents.

Women had a greater need for inhaled medication than men, a trend that has been observed in other countries (37). While literature on gender differences in medication adherence is inconclusive, it has been suggested that women may have a better understanding of the disease and therefore adhere to medication more effectively (42). It was found that patients living in least deprived areas had significantly higher Necessity scores. This could be attributed to the fact that these patients have probably easier access to healthcare resources and are more aware of the importance of their medication. But future studies collecting direct socio-economic data (*e.g.*, family income) are needed to validate this preliminary finding. Patients recruited in primary care had significantly lower Necessity and Concern scores, while still maintaining a positive Necessity-Concerns differential. In a previous study comparing beliefs across specialties, no differences were observed for the necessity score, but for the concerns score the results are consistent with our study, with patients recruited by primary care physicians showing lower concerns (21). This may be because primary care settings often deal with less severe or better controlled cases of asthma, where patients may have milder symptoms or more stable conditions. The Portuguese primary care system has been found to manage chronic conditions more effectively than other countries, being among the ones with lowest rates of hospital admission for asthma in the European Union (43). Conversely, patients with more severe or uncontrolled asthma are typically treated in secondary care settings. Patients referred to secondary care settings may have already encountered challenges with asthma management in primary care. This prior experience could impact on their beliefs about medication, potentially leading to higher concerns or a stronger perception of necessity.

Although asthma control and associated impact on the patients' daily lives could influence their perceptions of the necessity and concerns regarding medication, there were no differences found in the Necessity and Concerns scores regarding GINA, CARAT scores or exacerbations history.

The latest GINA guidelines recommend using a single inhaler for both maintenance and reliever therapy in adolescents and adults, containing long-acting beta$_2$ agonist (LABA) and low-dose inhaled corticosteroid (ICS) (3). This approach is more effective than using separate inhalers for maintenance and reliver therapy. This study

found that patients prescribed with more than one inhaler had significantly higher Necessity scores than patients using only one inhaler. Although, in our analysis, it is not specified what kind of inhaler is used (short-acting beta$_2$ agonist (SABA) or ICS/LABA or other) and future studies could clarify this link. It might be the case that patients with asthma may be more aware of the necessity of medication when treatment regimens are more complex or when their asthma is more severe. However, complexity of treatment can also impact patient adherence. Healthcare professionals should address this topic and aim to simplify treatment regimens, by following the most recent guidelines.

Previous studies have shown that, while patients with strong beliefs about necessity of medication are more likely to be completely adherent, patients who have higher levels of concern are more likely to have lower adherence to medication (13, 14, 44, 45). The notably higher Necessity scores observed in individuals classified as highly adherent by the VAS align with previous research. Horne *et al.* reported that patients with strong necessity beliefs were 1.7 times more likely to exhibit adherence to asthma medication (13). However, regarding concerns, we were able to find differences using MMA but not the VAS on medication adherence. This may be explained by the simplicity of the self-reported questionnaires used in our study in comparison with more complex patient-reported outcome measures, such as the eight item Morisky Medication Adherence Scale and the Medication Adherence Rating Scale, that try to identify the circumstances/situations related to adherence behavior (44). It is known that self-reported questionnaires may also lead to overestimation of true adherence by respondents. This can also be attributed to the telephone application of questionnaires, which differed from the data collection strategy in previous studies (mostly, self-report in paper versions). In paper self-reports respondents typically have a greater sense of anonymity and may feel less pressure to provide socially desirable responses.

Our study holds several strengths that should be acknowledged. Firstly, it is a multicenter investigation that enrolled participants across various age groups, including and comparing both adults and adolescents with asthma, which is not found in previous articles. Secondly, the inclusion of a large and diverse sample recruited from both primary and secondary care settings in real-world practice enhances the prominence of this study, providing insights into the beliefs of patients encountered in routine clinical practice. The use of a validated tool, such as the BMQ-Specific allows for a multifaceted evaluation of patients' beliefs. This study has also some limitations that should be acknowledged. The study included a large percentage of patients with self-reported uncontrolled asthma (assessed with CARAT), and concerns and beliefs about medication may be perceived differently by this group. Therefore, the findings from this analysis may not be directly applicable to individuals who have good asthma control. Nevertheless, it should be noted that the studied population was obtained through a convenience sample, which may limit the generalizability of the results, as it only includes patients seeking medical care, who therefore may have higher concerns or poorer disease control. Future analysis could include a consecutive patient sample and investigate the role of disease duration and severity. Another limitation can be the fact that beliefs were collected through telephone interview, which may introduce bias, as respondents may provide socially desirable or inaccurate information unconsciously In addition, this is a cross-sectional analysis, which can be limiting given that beliefs about treatment and adherence patterns are subject to evolution over time. Our study may not fully capture the dynamic nature of these factors and their interplay, but it can be done in future research through longitudinal investigations.

This study contributes to the understanding of patients' beliefs about inhaled medication for asthma in a large, real-world population in Portugal. A predominantly positive perception of inhaled asthma medication necessity was found, although ambivalence or indifference exist in about 1/5 of patients. By addressing age-specific disparities and considering patient-related factors, the findings pave the way for targeted interventions and improved patient outcomes in asthma care. Future research should include longitudinal analysis and adherence objective measures to provide a more dynamic understanding of medication adherence, treatment beliefs, and asthma control over time.

Fundings

This study is a secondary analysis of the mINSPIRE project financed by national funds through the Foundation for Science and Technology (PTDC/MEC-OUT/29130/2017) and co-financed by Operational Programme "Competitiveness and Internationalization" (COMPETE 424 2020), PORTUGAL 2020 from European Regional Development Fund - FEDER (POCI-01-0145-425 FEDER-029130).

Contributions

AMP, RuAl, RiAm, JAF, CJ: conceptualization. CN, AMC, AMP, JAF, CJ: data collection. CN, CJ: formal analysis. CN: writing – original draft. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. The Global Asthma Report 2022. Int J Tuberc Lung Dis. 2022;26(Supp 1):1-104. doi: 10.5588/ijtld.22.1010.
2. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study

2019. Lancet. 2020;396(10258):1204-1222. doi: 10.1016/S0140-6736(20)30925-9.
3. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2023. Available at: https://ginasthma.org/wp-content/uploads/2023/07/GINA-2023-Full-report-23_07_06-WMS.pdf.
4. Adherence to Long-Term Therapies: Evidence for Action. Geneve, Switzerland: World Health Organization; 2003.
5. Bårnes CB, Ulrik CS. Asthma and adherence to inhaled corticosteroids: current status and future perspectives. Respir Care. 2015;60(3):455-68. doi: 10.4187/respcare.03200.
6. Kaplan A, Price D. Treatment Adherence in Adolescents with Asthma. J Asthma Allergy. 2020;13:39-49. doi: 10.2147/JAA.S233268.
7. Sims EJ, Price D, Haughney J, Ryan D, Thomas M. Current control and future risk in asthma management. Allergy Asthma Immunol Res. 2011;3(4):217-25. doi: 10.4168/aair.2011.3.4.217.
8. Mäkelä MJ, Backer V, Hedegaard M, Larsson K. Adherence to inhaled therapies, health outcomes and costs in patients with asthma and COPD. Respir Med. 2013;107(10):1481-90. doi: 10.1016/j.rmed.2013.04.005.
9. Murphy AC, Proeschal A, Brightling CE, Wardlaw AJ, Pavord I, Bradding P, et al. The relationship between clinical outcomes and medication adherence in difficult-to-control asthma. Thorax. 2012;67(8):751-3. doi: 10.1136/thoraxjnl-2011-201096.
10. Stern L, Berman J, Lumry W, Katz L, Wang L, Rosenblatt L, et al. Medication compliance and disease exacerbation in patients with asthma: a retrospective study of managed care data. Ann Allergy Asthma Immunol. 2006;97(3):402-8. doi: 10.1016/S1081-1206(10)60808-3.
11. Boulet LP, Vervloet D, Magar Y, Foster JM. Adherence: the goal to control asthma. Clin Chest Med. 2012;33(3):405-17. doi: 10.1016/j.ccm.2012.06.002.
12. Horne R. Compliance, adherence, and concordance: implications for asthma treatment. Chest. 2006;130(1 Suppl):65S-72S. doi: 10.1378/chest.130.1_suppl.65S.
13. Horne R, Chapman SC, Parham R, Freemantle N, Forbes A, Cooper V. Understanding patients' adherence-related beliefs about medicines prescribed for long-term conditions: a meta-analytic review of the Necessity-Concerns Framework. PLoS One. 2013;8(12):e80633. doi: 10.1371/journal.pone.0080633.
14. Horne R, Weinman J. Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. J Psychosom Res. 1999;47(6):555-67. doi: 10.1016/s0022-3999(99)00057-4.
15. Horne R, Weinman J. Self-regulation and Self-management in Asthma: Exploring The Role of Illness Perceptions and Treatment Beliefs in Explaining Non-adherence to Preventer Medication. Psychol Health. 2002;17(1):17-32. doi: 10.1080/08870440290001502.
16. Horne R, Weinman J, Hankins M. The beliefs about medicines questionnaire: The development and evaluation of a new method for assessing the cognitive representation of medication. Psychol Health. 1999;14(1):1-24. doi: 10.1080/08870449908407311.
17. Menckeberg TT, Bouvy ML, Bracke M, Kaptein AA, Leufkens HG, Raaijmakers JA, et al. Beliefs about medicines predict refill adherence to inhaled corticosteroids. J Psychosom Res. 2008;64(1):47-54. doi: 10.1016/j.jpsychores.2007.07.016.
18. Van Steenis M, Driesenaar J, Bensing J, Van Hulten R, Souverein P, Van Dijk L, et al. Relationship between medication beliefs, self-reported and refill adherence, and symptoms in patients with asthma using inhaled corticosteroids. Patient Prefer Adherence. 2014;8:83-91. doi: 10.2147/PPA.S44185.
19. Kosse RC, Koster ES, Kaptein AA, de Vries TW, Bouvy ML. Asthma control and quality of life in adolescents: The role of illness perceptions, medication beliefs, and adherence. J Asthma. 2020;57(10):1145-1154. doi: 10.1080/02770903.2019.1635153.
20. Clifford S, Barber N, Horne R. Understanding different beliefs held by adherers, unintentional nonadherers, and intentional nonadherers: application of the Necessity-Concerns Framework. J Psychosom Res. 2008;64(1):41-6. doi: 10.1016/j.jpsychores.2007.05.004.
21. Chan AHY, Katzer CB, Pike J, Small M, Horne R. Medication beliefs, adherence, and outcomes in people with asthma: The importance of treatment beliefs in understanding inhaled corticosteroid nonadherence-a retrospective analysis of a real-world data set. J Allergy Clin Immunol Glob. 2022;2(1):51-60. doi: 10.1016/j.jacig.2022.09.006.
22. Koster ES, Philbert D, Winters NA, Bouvy ML. Adolescents' inhaled corticosteroid adherence: the importance of treatment perceptions and medication knowledge. J Asthma. 2015;52(4):431-6. doi: 10.3109/02770903.2014.979366.
23. Jácome C, Guedes R, Almeida R, Teixeira JF, Pinho B, Vieira-Marques P, et al. mINSPIRERS - Estudo da exequibilidade de uma aplicação móvel para medição e melhoria da adesão à medicação inalada de controlo em adolescentes e adultos com asma persistente. Protocolo de um estudo observacional multicêntrico. Revista Portuguesa de Imunoalergologia. 2018;26:47-61.
24. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg. 2014;12(12):1500-24. doi: 10.1016/j.ijsu.2014.07.014.
25. Ribeiro AI, Mayer A, Miranda A, Pina MF. The Portuguese Version of the European Deprivation Index: An Instrument to Study Health Inequalities. Acta Med Port. 2017;30(1):17-25. doi: 10.20344/amp.7387.
26. Vieira RJ, Sousa-Pinto B, Cardoso-Fernandes A, Jácome C, Portela D, Amaral R, et al. Control of Allergic Rhinitis and Asthma Test: A systematic review of measurement properties and COSMIN analysis. Clin Transl Allergy. 2022;12(9):e12194. doi: 10.1002/clt2.12194.
27. Fonseca JA, Nogueira-Silva L, Morais-Almeida M, Azevedo L, Sa-Sousa A, Branco-Ferreira M, et al. Validation of a questionnaire (CARAT10) to assess rhinitis and asthma in patients with asthma. Allergy. 2010;65(8):1042-8. doi: 10.1111/j.1398-9995.2009.02310.x.
28. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986;24(1):67-74. doi: 10.1097/00005650-198601000-00007.
29. Salgado T, Marques A, Geraldes L, Benrimoj S, Horne R, Fernandez-Llimos F. Cross-cultural adaptation of The Beliefs about Medicines Questionnaire into Portuguese. Sao Paulo Med J. 2013;131(2):88-94. doi: 10.1590/s1516-31802013000100018.
30. Jácome C, Pereira AM, Almeida R, Ferreira-Magalhaes M, Couto M, Araujo L, et al. Patient-physician discordance in assessment of adherence to inhaled controller medication: a cross-sectional analysis of two cohorts. BMJ Open. 2019;9(11):e031732. doi: 10.1136/bmjopen-2019-031732.
31. Rozban Y, Devilliers H, Hié M, Micheline P, Mathian A, Cohen-Aubart F, et al. FRI0336 Belief about medicine questionnaire predicts therapeutic adhesion to hydroxychloroquine during systemic lupus erythematosus. Ann Rheum Dis. 2018;77(Suppl 2):704. doi: 10.1136/annrheumdis-2018-eular.5675.
32. Pedersini R, Vietri J. Comparison of the 4-item and 8-item morisky medication adherence scale in patients with type 2 diabetes. Value Health. 2014;17(3):A183. doi: 10.1016/j.jval.2014.03.1066.

33. Delgado AB, Lima ML. Contributo para a validação concorrente de uma medida de adesão aos tratamentos. Psicologia, Saúde Doenças. 2001;2(2):81-100.
34. Humenberger M, Horner A, Labek A, Kaiser B, Frechinger R, Brock C, et al. Adherence to inhaled therapy and its impact on chronic obstructive pulmonary disease (COPD). BMC Pulm Med. 2018;18(1):163. doi: 10.1186/s12890-018-0724-3.
35. Bozek A, Jarzab J. Adherence to asthma therapy in elderly patients. J Asthma. 2010;47(2):162-5. doi: 10.3109/02770900903497204.
36. Lycett H, Wildman E, Raebel EM, Sherlock JP, Kenny T, Chan AHY. Treatment perceptions in patients with asthma: Synthesis of factors influencing adherence. Respir Med. 2018;141:180-189. doi: 10.1016/j.rmed.2018.06.032.
37. Chapman S, Dale P, Svedsater H, Stynes G, Vyas N, Price D, et al. Modelling the effect of beliefs about asthma medication and treatment intrusiveness on adherence and preference for once-daily vs. twice-daily medication. NPJ Prim Care Respir Med. 2017;27(1):61. doi: 10.1038/s41533-017-0061-7.
38. Han Y-Y, Gutwein A, Apter A, Celedón JC. Health Literacy and Asthma: An Update. J Allergy Clin Immunol. 2023. doi: 10.1016/j.jaci.2023.12.007.
39. Orrell-Valente JK, Jarlsberg LG, Hill LG, Cabana MD. At what age do children start taking daily asthma medicines on their own? Pediatrics. 2008;122(6):e1186-92. doi: 10.1542/peds.2008-0292.
40. De Simoni A, Horne R, Fleming L, Bush A, Griffiths C. What do adolescents with asthma really think about adherence to inhalers? Insights from a qualitative analysis of a UK online forum. BMJ Open. 2017;7(6):e015245. doi: 10.1136/bmjopen-2016-015245.
41. Morton RW, Everard ML, Elphick HE. Adherence in childhood asthma: the elephant in the room. Arch Dis Child. 2014;99(10):949-53. doi: 10.1136/archdischild-2014-306243.
42. Mahmoodi H, Jalalizad Nahand F, Shaghaghi A, Shooshtari S, Jafarabadi MA, Allahverdipour H. Gender Based Cognitive Determinants Of Medication Adherence In Older Adults With Chronic Conditions. Patient Prefer Adherence. 2019;13:1733-1744. doi: 10.2147/PPA.S219193.
43. OECD/European Observatory on Health Systems and Policies (2021), Portugal: Country Health Profile 2021, State of Health in the EU, OECD Publishing, Paris. doi: 10.1787/8f3b0171-en.
44. Alhewiti A. Adherence to Long-Term Therapies and Beliefs about Medications. Int J Family Med. 2014;2014:479596. doi: 10.1155/2014/479596.
45. Brandstetter S, Finger T, Fischer W, Brandl M, Böhmer M, Pfeifer M, et al. Differences in medication adherence are associated with beliefs about medicines in asthma and COPD. Clin Transl Allergy. 2017;7:39. doi: 10.1186/s13601-017-0175-6.

LETTER TO THE EDITOR
EDRA
Eur Ann Allergy Clin Immunol Vol 58, N.6, 279-282, 2026

Denis Vincent[1], Arije Ghanam[2]

Transitioning from nonspecific therapy to berotralstat in hereditary angioedema type I: real world data from three patients within the same family

[1]Department of Asthma, Allergology and Internal Medicine, University Hospital Carémeau, Nîmes, France
[2]KininBIO, Grenoble, France

Key words

Hereditary angioedema; androgens; tranexamic acid; berostraltat; switch.

Corresponding author
Denis Vincent
Department of Asthma, Allergology and Internal Medicine
University Hospital Carémeau
Place du Pr. Robert Debré
30029 Nîmes, France
ORCID: N/A
E-mail: Denis.vincent@umontpellier.fr

Doi
10.23822/EurAnnACI.1764-1489.391

To the Editor

hereditary angioedema (HAE) is a rare disorder characterized by recurrent episodes of edema affecting primarily subcutaneous tissues (face, upper or lower extremities, genitals), abdominal organs (stomach, intestines, bladder), and upper airways (larynx, tongue) (1, 2). The edema can be life threatening when affecting the upper airways.

The majority of HAE cases are caused by variants of the gene *SERPING1* encoding for C1 inhibitor (C1INH), resulting in deficiency or dysfunction of C1INH (HAE-C1INH-Type 1 or 2 respectively). In HAE-C1INH, angioedema is mediated by dysregulation of the kallikrein-kinin system, resulting in localized overproduction of bradykinin, causing increased vascular permeability.

Disease management is based on the following triad: on-demand treatment of crises, short-term prophylaxis, and long-term prophylaxis (LTP) (3). Historically, non-specific treatments have been employed for LTP such as danazol that promotes increased hepatic synthesis of C1INH, associated with numerous adverse effects (weight gain, hypertension, dyslipidaemia, acne, irregular menstrual cycles, increased CPK enzymes and decreased libido), or tranexamic acid (TXA), an antifibrinolytic agent inhibiting plasmin production that showed limited efficacy (4).

Recently, specific plasma kallikrein inhibitors demonstrated their effectiveness in reducing HAE attacks, 2 of them being indicated as first-line LTP(3), namely lanadelumab, a humanized monoclonal antibody administered subcutaneously every 2-4 weeks, and berotralstat, an oral plasma kallikrein inhibitor administered once daily (5).

Due to the recent availability of berotralstat and the rarity of the disease, real-world data remain limited. Moreover, the transition modalities from previous therapies to berotralstat have not yet been precisely described: in clinical studies, patients underwent a wash-out period before initiating berotralstat therapy (5). In this case series, we describe the transition process in a real-world clinical setting.

Three patients from the same family were transitioned to berostraltat at the same hospital. Written consent was obtained prior to data collection. Clinical monitoring was done during outpatient clinic visits during which number of attacks, possible side

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

effects, quality of life, and therapeutic compliance using the 8-item Morisky Medication Adherence Scale (MMAS-8) (6) were recorded.

The 3 patients (2 women and 1 man) had been diagnosed with HAE-C1INH-Type I 9 years ago, with various disease phenotypes as regards frequency and severity of crises (**table I**). Patients 1 and 2 had been treated with danazol as LTP for 3 years. Concerns regarding long-term tolerability and/or persistence of severe attacks led to the decision to switch to berotralstat (**table I**). Moreover, Patient 1 wished to avoid injectable treatments. Patient 3 was treated with TXA as on-demand treatment of attacks without LTP. However, its effectiveness had been decreasing since puberty (occurrence of severe attacks requiring icatibant).

Switching modalities are detailed in **figure 1**.

For Patient 1, switch to berotralstat occurred over a brief period (1 month) since the patient was already receiveing the lowest possible dosage of danazol (200 mg/week). The one-month overlapping period was implemented to reach the berotralstat's steady-state, which typically ranges from 6 to 12 days (7). Patient 2, being on a higher danazol dosage, required a 3-month tapering of danazol while initiating berotralstat concurrently, at the end of which danazol were completely discontinued. Patient 3, solely treated with on-demand treatments, did not require a transition period. After switch, no attack was reported during 6 (Patient 3) to 9 months (Patients 1 and 2) of follow-up, which was associated to improved patients' satisfaction. Berotralstat exhibited superior tolerability compared to danazol, with no reported side effects. All patients achieved a MMAS-8 score of 8, reflecting a high level of adherence to treatment.

Androgens and TXA are no longer recommended for LTP due to adverse safety profile and insufficient efficacy respectively(3). The international guidelines also highlighted that, on top of achieving complete disease control, the goal of LTP is to normalize patients' lives by minimizing treatment burden and adverse effects (3).

There is no precise recommendation on how transitioning from danazol to berotralstat, but abrupt discontinuation of androgens should be avoided (7). Three options have been described (8): tapering danazol after initiation of new treatment, tapering danazol followed by a washout period, or initiating new therapy while tapering danazol. We chose the third option to avoid attacks during the transition period, which may have deleterious psychological impact in patients. Following this method, we observed successful transitions from danazol and TXA to berotralstat in our 3 patients.

Close monitoring and regular communication with patients during the transition period were instrumental in achieving optimal outcomes. HAE is characterized by the absence of validated biomarkers for disease monitoring. Consequently, disease evaluation solely relies on clinical examination and patient's testimonies. Hence, it is crucial to involve the patient in therapeutic decisions and to foster an environment of discussion during or outside of consultations.

Berotralstat therapy requires daily tablet intake. Therapeutic adherence represents a significant public health challenge. The JALMA study indicated that 25% of prescribed medications are not taken by patients, a common negligence among individuals with life-threatening conditions, leading to 12,000 deaths and 100,000 hospitalizations each year (9). Therefore, every effort should be made to improve compliance including therapeutic education, in-depth discussions with patients to understand reasons for poor compliance (fear of side-effects, poor understanding of the disease, erroneous beliefs on drugs), detection and early management of possible side-effects. It is essential to create a relationship of trust with the patient to ensure straightforward communication. Patients' preferences and constraints should be questioned to choose treatment, in particular administration route and frequency of administration. In HAE, it has been made possible thanks to the availability of new options for LTP. In our

Figure 1 *- Switching modalities from previous treatment to berotralstat.*

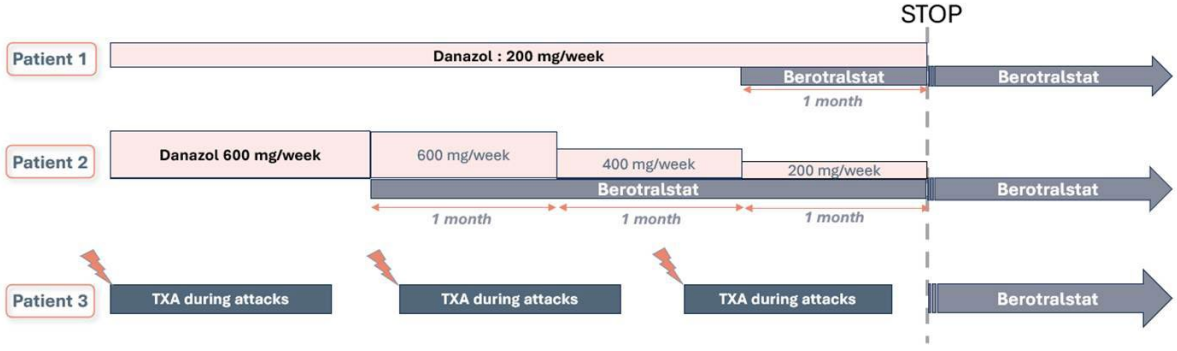


Table I *- Demographic characteristics and previous treatments.*

	Patient 1 (index case's cousin)	**Patient 2 (index case)**	**Patient 3 (index case's daughter)**
Sex	Female	Male	Female
Age at transition to berostraltat (years)	46	58	20
Weight (kgs)	61	72	57
Comorbidities	Hashimoto's thyroiditis	None	None
Concomitant therapies	None	None	Desogestrel
Age at HAE diagnosis	37 years	49 years	11 years
Mutation	*SERPING1* c.1353_1354delGA delGA/wt		
C1INH (antigenic level // function)	47 mg/L // 3.8 U/mL	37 mg/L // 2.5 U/mL	52 mg/L // < 2 U/mL
Attacks description*	Extremities (arm, hand, foot). Not severe, disappear spontaneously.	Laryngeal oedema triggered by local trauma	Mainly facial oedema and abdominal pain.
Attack rate before switch	0 (controlled with danazol)	1.5/year (severe attacks)	1 severe attack every 1 or 2 years
Treatment before switch			
LTP	Danazol	Danazol	None
Treatment of attacks	Icatibant	Icatibant	Tranexamic acid (TXA) ± icatibant
Dosage	200 mg/week	600 mg/week	2 g orally during attacks
Reason for the switch	Poor tolerance Concerns about long-term use in a woman and wish not to use injectables	Poor efficacy, burden of treatment and poor tolerance (muscular pain, increased CPK and liver transaminases)	Lack of efficacy TXA efficacy deteriorated during puberty, with recurrent oedema and fainting.
Therapeutic goals	1) Maintain disease control 2) Avoid side effects of danazol	1) Avoid side effects of danazol 2) Maintain disease control 3) Improve quality of life	1) Maintain disease control

*Under danazol LTP for patients 1 and 2, under ODT by TXA for patient 3.

series, all three patients reported a high level of treatment adherence according to the MMAS-8 questionnaire.

This publication has several limitations, namely the small sample size, the absence of validated scales to assess quality of life and disease control, and the short duration of follow-up.

This case series highlights the benefits of new treatments such as berotralstat in the management of HAE, providing improved tolerability and effective disease control. It emphasizes the importance of patient-centered care throughout the transition process. Further research and long-term studies are needed to validate these findings and optimize HAE management strategies.

Fundings

Financial support was provided by Biocryst for medical writing.

Contributions

DV: writing – original draft, conceptualization, project administration. AG: writing – review & editing, data curation, formal analysis.

Conflict of interests

DV received travel grants and expert fees from Biocryst. AG received fees from Biocryst for medical writing.

References

1. Launay D, Bouillet L, Boccon-Gibod I, Trumbic B, Gobert D; Centres coordonnateur et constitutifs CREAK, et al. Mise au point sur les angiœdèmes héréditaires et leurs nouvelles thérapeutiques [Hereditary

angioedema and its new treatments: An update]. Rev Med Interne. 2023;44(7):344-353. French. doi: 10.1016/j.revmed.2023.01.020.
2. Maurer M, Aygören-Pürsün E, Banerji A, Bernstein JA, Balle Boysen H, Busse PJ, et al. Consensus on treatment goals in hereditary angioedema: A global Delphi initiative. J Allergy Clin Immunol. 2021;148(6):1526-32. doi: 10.1016/j.jaci.2021.05.016.
3. Maurer M, Magerl M, Betschel S, Aberer W, Ansotegui IJ, Aygoren-Pursun E, et al. The international WAO/EAACI guideline for the management of hereditary angioedema-The 2021 revision and update. Allergy. 2022;77(7):1961-90. doi: 10.1111/all.15214.
4. Christiansen SC, Davis DK, Castaldo AJ, Zuraw BL. Pediatric Hereditary Angioedema: Onset, Diagnostic Delay, and Disease Severity. Clin Pediatr (Phila). 2016;55(10):935-42. doi: 10.1177/0009922815616886.
5. Zuraw B, Lumry WR, Johnston DT, Aygoren-Pursun E, Banerji A, Bernstein JA, et al. Oral once-daily berotralstat for the prevention of hereditary angioedema attacks: A randomized, double-blind, placebo-controlled phase 3 trial. J Allergy Clin Immunol. 2021;148(1):164-72.e9. doi: 10.1016/j.jaci.2020.10.015.
6. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986;24(1):67-74. doi: 10.1097/00005650-198601000-00007.
7. European Medicines Agency. Orladeyo SmPC. Available at: https://www.ema.europa.eu/en/documents/product-information/orladeyo-epar-product-information_en.pdf.
8. Gurugama P, Launay D. Transitioning Patients From Second- to First-Line Prophylaxis in Hereditary Angioedema. EMJ. 2024;91(9):22-7. doi: 10.33590/emj/10307646.
9. JALMA. Les enjeux de l'observance en France, cinq propositions pour renforcer l'observance des produits de santé. 2014. Available at: https://www.lemoniteurdespharmacies.fr/ressources/upload/imgnewspha/depot/synthese-observatoire-observance.pdf.

EDRA Eur Ann Allergy Clin Immunol Vol 58, N.6, 283-285, 2026

Riccardo Asero

Severe chronic spontaneous urticaria with low total IgE levels: is the response to omalizumab always delayed or absent?

Allergology Clinic, Clinica San Carlo, Paderno Dugnano, Milan, Italy

Key words

Chronic spontaneous urticaria; IgE; omalizumab.

Corresponding author
Riccardo Asero
Allergology Clinic
Clinica San Carlo
via Ospedale 21
20037 Paderno Dugnano, Milan, Italy
ORCID: 0000-0002-8277-1700
E-mail: r.asero@libero.it

Doi
10.23822/EurAnnACI.1764-1489.360

To the Editor,

in patients with severe chronic spontaneous urticaria (CSU) omalizumab represents the treatment of choice if there is no response to H1 antihistamines at higher than licensed dose (1). This anti-IgE mAb is able to induce a rapid drop in UAS7 levels in about 70% of patients and a slower but equally good response over 3-4 months in further 15%. Many studies found a prognostic relationship between baseline total IgE and clinical response to omalizumab (2-7). The threshold level predicting a non-response or a late response has been detected at < 40 IU/ml in some studies (8) and at 18 IU/ml or less in others (2, 3, 9). However, based on the clinical response to omalizumab, not all severe CSU patients with low IgE levels seem to have an autoimmune, IgG-mediated (type IIb) disease (10). This retrospective study focused on severe CSU with low total IgE levels and its response to omalizumab.
Eighty-six consecutive patients (M/F 19/67) with severe CSU (UAS7 > 30) and baseline total IgE levels (measured by commercial ELISA, Thermo Fisher Scientific, Waltham, MA, USA) < 40 IU/ml unresponsive to second generation antihistamines at 3x the licensed dosage and treated with omalizumab 300 mg/month for at least 3 months were studied.

The mean age was 50.5 years (range 7-76), and the disease duration was 41.5 (2-600) months. An UAS7 drop > 50% one month after the first administration were considered as early responders. A drop > 50% from baseline within one month after the 3rd administration was considered as late responders. The absence of any change in UAS7 at month 4 was considered a non-response.
The following baseline parameters were assessed: 1) Atopic status (detected by SPT with a large panel of seasonal and perennial airborne allergens [Lofarma, Milano, Italy]; reactivity to at least one allergen was considered a marker of atopy). 2) D-dimer (measured by ELISA [Hyphen BioMed, Neuville sur Oise, France] and considering levels below 500 ng/ml as normal). 3) Thyroid peroxidase (TPO) IgG autoantibodies (measured by ELISA [Thermo Fisher Scientific]; values > 60IU/ml were considered positive. 4) C reactive protein (CRP) (measured by a sandwich immunoassay [Hyphen BioMed]). Proportions were compared by chi-square test with Yates' correction. The internal review board of the Clinic approved this retrospective, observational, anonymous study.
Based on total IgE levels, patients were stratified into 4 subgroups (A, total IgE levels 1-9 IU/ml [n= 28]; B, IgE 10-19 IU/

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

Table I *- Clinical response to omalizumab and clinical and serological variables in patients with severe CSU stratified by total IgE levels.*

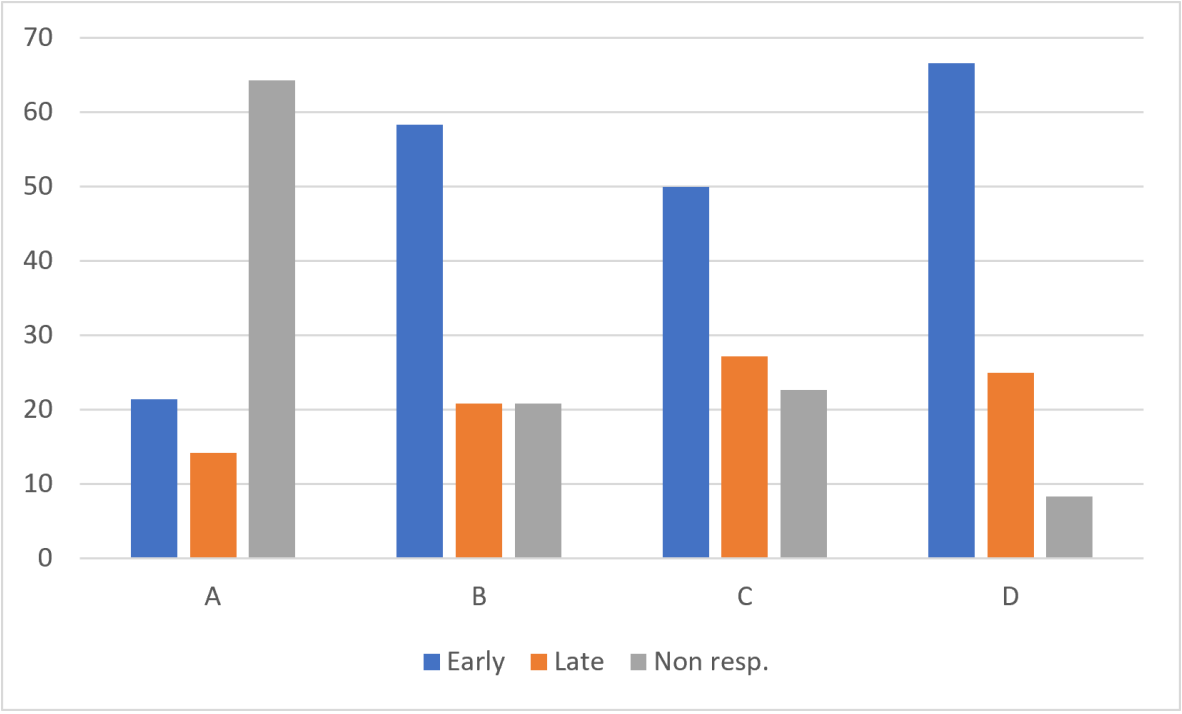
Subgroup	Early response	Late response	Non-response	Atopic status	Elevated CRP	Thyroid autoimmunity	Elevated D-dimer
A (n = 28)	6 (21.4%)*	4 (14.3%)	18 (64.3%)**	2 (7.1%)	9 (32%)	10/26 (38.5%)	13/24 (54.1%)
B (n = 24)	14 (58.3%)	5 (20.8%)	5 (20.8%)	5 (20.8%)	5 (20.8%)	3/23 (13%)	8/17 (47.1%)
C (n = 22)	11 (50%)	6 (27.2%)	5 (22.7%)	3 (13.6%)	4 (18.2%)	10/22 (45.4%)	7/20 (35%)
D (n = 12)	8 (66.6%)	3 (25%)	1 (8.3%)	3 (25%)	1 (8.3%)	1/11 (9.1%)	4/11 (36%)

*p < 0.005 for subgroup A *vs* B+C+D; **p < 0.001 for subgroup A *vs* B+C+D.

ml [N=24]; C, IgE 20-29 IU/ml [n=22]; and D, IgE 30-39 IU/ml [n=12]). Omalizumab response is shown in **table I** and **figure 1**. Subgroup A showed the lowest prevalence of early responders (21.4%; p < 0.005 *vs* subgroups B-D) and the highest prevalence of non-responders (64.3%; p < 0.001 *vs* subgroups B-D). Notably, the proportion of unresponsive patients decreased dramatically from subgroup A to D (**figure 1**). Prevalence of atopic status, thyroid autoimmunity, positive CRP, and elevated D-dimer are shown in **table 1**. No statistically significant difference was found between the 4 subgroups. Since all non-responders were shifted to cyclosporin treatment and responders are still being treated with omalizumab, there were no sufficient data to assess the effect of omalizumab discontinuation and disease relapse.

This study found that many patients with severe CSU and low total IgE levels respond rapidly to omalizumab. Such proportion exceeded 20% in the subgroup with the lowest IgE levels and ranged between 50% and 68% in the three remaining subgroups. This suggests that also patients with probable type I (autoallergic) CSU may show very low total IgE levels. Mechanisms other than direct IgE binding (including down-regulation of IgE receptors on mast cells and eosinophil apoptosis) may contribute to the efficacy of omalizumab. However, such mechanisms are considered to be associated with a delayed response to omalizumab which was similar in the four subgroups studied here. Although – not surprisingly – the early response rate to omalizumab in these specific patients was lower than in the general population with CSU, this

Figure 1 *- Omalizumab response in the 4 subgroups of patients with severe CSU.*



study suggests that total IgE cannot be considered an absolute predictive marker of response to this drug. In view of its very limited toxicity and reasonable cost, omalizumab should remain stably the third step of CSU management before novel treatments such as Bruton's tyrosine kinase (BTK) inhibitors or anti-Stem cell factor (SCF) mAb that will appear in the next future can be considered.

Fundings

None.

Contributions

RA contributed entirely to the work.

Conflict of interests

The author declares that he has no conflict of interests.

References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022;77(3):734-766. doi: 10.1111/all.15090.
2. Straesser MD, Oliver E, Palacios T, Kyin T, Patrie J, Borish L, et al. Serum IgE as an immunological marker to predict response to omalizumab treatment in symptomatic chronic urticaria. J Allergy Clin Immunol Pract. 2017;6(4):1386-1388. doi: 10.1016/j.jaip.2017.10.030.
3. Ertas R, Ozyurt K, Atasoy M, Hawro T, Maurer M. The clinical response to omalizumab in chronic spontaneous urticaria patients is linked and predicted by IgE levels and their change. Allergy. 2018;73(3):705-12. doi: 10.1111/all.13345.
4. Marzano AV, Genovese G, Casazza G, Fierro MT, Dapavo P, Crimi N, et al. Predictors of response to omalizumab and relapse in chronic spontaneous urticaria: a study of 470 patients. J Eur Acad Dermatol Venereol. 2019;33(5):918-924. doi: 10.1111/jdv.15350.
5. Weller K, Ohanyan T, Hawro T, Ellrich A, Sussman G, Koplowitz J, et al. Total IgE levels are linked to the response of chronic spontaneous urticaria patients to omalizumab. Allergy. 2018;73(12):2406–2408. doi: 10.1111/all.13586.
6. Asero R, Ferrucci S, Casazza G, Marzano AV, Cugno M. Total IgE and atopic status in patients with severe chronic spontaneous urticaria unresponsive to omalizumab treatment. Allergy. 2019;74(8):1561-1563. doi: 10.1111/all.13754.
7. Keller L, Perera EK, Bindon B, Khatiwada A, Stitt JM, Dreskin SC. Total IgE as a biomarker of omalizumab response in chronic spontaneous urticaria: A meta-analysis. Allergy Asthma Proc. 2024;45(2):97-99. doi: 10.2500/aap.2024.45.230092.
8. Lee HY, Jeon HS, Jang JH, Lee Y, Shin YS, Nahm DH, et al. Predicting responses to omalizumab in antihistamine-refractory chronic urticaria: A real-world longitudinal study. J Allergy Clin Immunol Glob. 2024;3(2):100245. doi: 10.1016/j.jacig.2024.100245.
9. Asero R. Clinical variables of severe chronic spontaneous urticaria from total IgE standpoint: a retrospective study Eur Ann Allergy Clin Immunol. 2022;54(1):30-33. doi: 10.23822/EurAnnACI.1764-1489.191.
10. Kolkhir P, Muñoz M, Asero R, Ferrer M, Kocatürk E, Metz M, et al. Autoimmune chronic spontaneous urticaria. J Allergy Clin Immunol. 2022;149(6):1819-1831. doi: 10.1016/j.jaci.2022.04.010.

LETTER TO THE EDITOR
EDRA
Eur Ann Allergy Clin Immunol Vol 58, N.6, 286-288, 2026

Danilo Villalta[1], Mariaelisabetta Conte[1], Daniela Visentini[2], Matteo Furlani[3], Francesco Ellero[3], Roberta Metani[3], Fiorenza Pesente[2], Paolo Metus[4]

Redefining reference intervals for baseline serum tryptase: found the solution?

[1]Immunology and Allergy, S. Maria degli Angeli Hospital, ASFO, Azienda Sanitaria Friuli Occidentale (ASFO), Pordenone, Italy
[2]Institute of Clinical Pathology, Department of Laboratory Medicine, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy
[3]Department of Medicine (DMED), University of Udine, Udine, Italy
[4]Clinical Pathology, S. Maria degli Angeli Hospital, Azienda Sanitaria Friuli Occidentale (ASFO), Pordenone, Italy

Key words

Baseline serum tryptase; reference interval; upper reference limit; clinical decision limits.

Corresponding author
Danilo Villalta
S. Maria degli Angeli Hospital
Azienda Sanitaria Friuli Occidentale
via Montereale 24
33170 Pordenone, Italy
ORCID: 0000-0002-8865-4150
E-mail: villaltadanilo@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.398

To the Editor,

tryptase is a serine protease enzyme primarily expressed by mast cells (MCs) and, to a lesser extent, by basophils (1). The constitutive release of tryptase from MCs results in a baseline serum tryptase (BST) level, which remains relatively stable in individuals throughout life. Since BST levels correlate with mast cell load, persistently elevated levels are an important screening tool for the diagnosis of systemic mastocytosis. In contrast, a transient increase in serum tryptase occurs immediately after MC activation, for example, due to an allergic reaction.

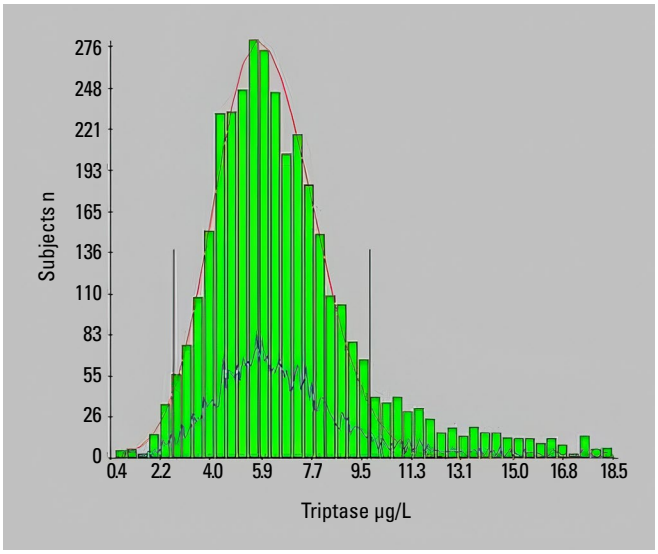
However, severe kidney damage, myeloid neoplasms, certain chronic inflammatory diseases, some chronic infections, and obesity can also cause elevated BST levels (2). Additionally, hereditary alpha tryptasemia (HαT), an autosomal dominant genetic trait characterized by an increased number of *TPSABI* gene copies encoding alpha tryptase, has recently been identified as the most prevalent cause of slightly to moderately elevated BST levels, affecting approximately 4% to 7.5% of the population (3).

Although serum tryptase measurement is performed worldwide using a single fluorescent enzyme immunoassay, there remains ongoing debate regarding the most appropriate reference interval (RI). The generally accepted RI suggested by the manufacturer, based on measuring tryptase levels in 126 'healthy' donors and determining the 95th percentile, is 1.0 (lower reference limit - LRL) to 11.4 µg/L (upper reference limit - URL). While the LRL is not clinically relevant, the URL is highly important for clinical decisions. However, it has been demonstrated that some individuals with systemic mastocytosis may have tryptase levels below 11.4 µg/L (4), indicating that the correct URL is lower than the 11.4 µg/L value proposed by the manufacturer, as the population used to determine the RI undoubtedly included individuals with HαT.

© 2026 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by Edra Media S.r.l. All rights reserved

Recently, experts from the European Competence Network on Mastocytosis (ECNM) and the American Initiative in Mast Cell Disease concluded that the "normal serum tryptase range" should be defined in asymptomatic controls, inclusive of individuals with HαT, since HαT *per se* should not be considered a disease-defining feature or pathology. Based on data from the literature, they concluded that the lower limit of BST, below which only a few HαT-positive cases are detected, is 8 µg/L. However, in daily practice, to avoid unnecessary referrals, investigations, and undue concerns in patients with HαT, the "normal range" of basal tryptase is up to 15 µg/L (2). Unfortunately, none of the previous studies in the literature established RIs following the gold standard direct method EP28-A3c (5), suggested by the CLSI (Clinical & Laboratory Standards Institute) and approved by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and by the World Health Organization (WHO). The main limitations of this standard method are that it is expensive, difficult, and time-consuming. An accepted alternative to the direct method is the indirect approach using big data previously generated in the laboratory and stored in the laboratory information system (LIS), referred to as data mining (6). Indirect methods are characterized by the fact that the reference subjects are not selected individually, and the RIs are statistically derived from the total distribution of unselected values. The aim of our study was to obtain the RI of BST using Kairisto's indirect approach, which is based on the assumption that most results produced by the laboratory derive from 'healthy' subjects and hypothesizes that the mode of the frequency distribution is the same for the whole dataset as it is for a 'healthy' reference population. We adopted Kairisto's procedure to correct and standardize the 10.332 tryptase determinations stored in two LIS (Pordenone and Udine, Italy). Only a single value per patient was used, usually the first one. Data were anonymized and elaborated using the GraphROC for Windows, version 2.0 statistical program (7), and mean, mode, LRL (2.5 percentile) and URL (97.5 percentile), were calculated, after the exclusion of outliers, with the use of the iterative ± 4SD method. Mean, mode, LRL and URL values of the whole studied population and divided by age and gender are shown in table 1 and the distribution of tryptase values in whole population in **figure 1**.

Figure 1 *- Distribution of tryptase values in the whole population of the study: 10,332 subjects.*



LRL and URL resulted 2,43 µg/L and 8,96 µg/L respectively, without significant difference between males (2,56 µg/L and 8,64 µg/L) and females (2,8 µg/L and 8,75 µg/L). On the contrary, there was a slight increase with age (**table I**).

The URL of BST obtained with Kairisto's indirect approach is very similar to the value previously proposed by Slot *et al.* (8), who used Bhattacharya's indirect approach on 5,248 measurements, and by the ECNM and the American Initiative in Mast Cell Disease as the "normal range where HαT is rather unlikely" (2). However, in clinical chemistry, the term "normal" range has not been used since Gräsbeck introduced the concept of RI, also known as the reference range (9). RI is a range of values, usually derived from "healthy" or disease-free individuals, to which results can be compared to aid interpretation. It should allow

Table I *- Mean, mode, lower reference limit (LRL) and upper reference limit (URL) obtained with the indirect Kairisto's approach evaluating 10,332 tryptase determinations from two different laboratories.*

	n	Outliers (n)	Mean (SD) µg/L	Mode µg/L	LRL µg/L	URL µg/L
All subjects	10,332	419	5.82 (2.42)	4.75	2.43	8.96
Male	3,699	161	6.04 (2.70)	4.92	2.56	8.64
Female	6,623	268	5.63 (2.26)	3.97	2.80	8.75
≤ 30 Years	2,143	100	4.64 (1.65)	3.67	2.44	7.10
31-60 Years	4,641	209	5.48 (2.04)	5.96	1.74	7.84
>60 years	3,495	163	6.82 (2.88)	5.73	2.70	9.80

the transformation of a number derived from an analytical measurement into clinically meaningful information. On the other hand, clinical decision limits (CDLs) may be an alternative to RIs. CDLs are cut-off values determined by clinical studies and associated with a specific clinical risk or outcome (6). The choice of a CDL is determined by the sensitivity and specificity of a test at that level. While RIs allow for comparison to a reference population, CDLs are linked to clinical outcomes. Regarding BST levels, the RI should be understood as the value obtained using a validated direct or indirect approach, whereas the limits proposed by the ECNM and the American Initiative in Mast Cell Disease should be considered CDLs. For BST, CDLs may be more appropriate than RIs in clinical practice, based on the expert panel's considerations.

In conclusion, the URL of BST obtained with an indirect approach using big data was 8.96 µg/L (ranging from 7.1 µg/L of the subjects < 30 years to 9.8 µg/L of the subject > 60 years). Future prospective studies will be necessary to define the sensitivity, specificity, and positive and negative likelihood ratios for the diagnosis of mastocytosis of the URL we proposed, as well as of the various CDLs suggested by the expert panel. Pending the results of such studies, in clinical practice it is advisable to follow the recommendations provided by the panel of experts.

Fundings

None.

Contributions

DanVill, MC, PM, DanVis: conceptualization, writing – original draft. FP, FE, MF, RM: data collection, formal analysis. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol. 2015;63(1):18-24. doi: 10.1016/j.molimm.2014.04.001.
2. Valent P, Hoermann G, Bonadonna P, Hartmann K, Sperr WR, Broesby-Olsen S, et al. The normal range of baseline tryptase should be 1 to 15 ng/mL and covers healthy individuals with HαT. J Allergy Clin Immunol Pract. 2023;11(10):3010-3020. doi: 10.1016/j.jaip.2023.08.008.
3. Lyons JJ, Sun G, Stone KD, Nelson C, Wisch L, O'Brien M, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. J Allergy Clin Immunol. 2014 May;133(5):1471-4. doi: 10.1016/j.jaci.2013.11.039.
4. Borer-Reinhold M, Haeberli G, Bitzenhofer M, Jandus P, Hausmann O, Fricker M, et al. An increase in serum tryptase even below 11.4 ng/mL may indicate a mast cell-mediated hypersensitivity reaction: a prospective study in Hymenoptera venom allergic patients. Clin Exp Allergy. 2011;41(12):1777-83. doi: 10.1111/j.1365-2222.2011.03848.x.
5. Boyd JC. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory: approved guidelines-Third Edition. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standard institute; 2008.
6. Timbrell NE. The Role and Limitations of the Reference Interval Within Clinical Chemistry and Its Reliability for Disease Detection. Br J Biomed Sci. 2024;81:12339. doi: 10.3389/bjbs.2024.12339.
7. Kairisto V, Poola A. Software for illustrative presentation of basic clinical characteristics of laboratory tests--GraphROC for Windows. Scand J Clin Lab Invest Suppl. 1995;222:43-60. doi: 10.3109/00365519509088450.
8. Slot MC, Claessen LHJ, Bons JAP, Menheere PPCA, Nieuwhof CMG, de Boer D. Tryptase reference ranges are age-dependent in a large population-based cohort. Allergy. 2022;77(9):2833-2834. doi: 10.1111/all.15369.
9. Gräsbeck R. The evolution of the reference value concept. Clin Chem Lab Med. 2004;42(7):692-7. doi: 10.1515/CCLM.2004.118.

Author Guidelines

European Annals of Allergy and Clinical Immunology will accept for publication suitable manuscripts dealing with the aetiology, diagnosis, and treatment of allergic and immunologic diseases. These might include the study of methods of controlling immunologic and allergic reactions, human and animal models of hypersensitivity and other aspects of basic and applied clinical allergy in its broadest sense. Papers reporting the results of drug trials will be considered. **European Annals of Allergy and Clinical Immunology** also publishes solicited and unsolicited review articles on subjects of topical interest to clinical and experimental allergy.

Manuscript

We request that all manuscripts should be submitted online through our web-based peer review system. Please go to:
https://www.editorialmanager.com/eaaci/default2.aspx.
Submitted contributions are accepted for publication on the basis of scientific interest and relevance, at the final discretion of the Editors in Chief, who will have blinded written evaluations from at least two anonymous reviewers.

Once a manuscript has been accepted for publication, Authors will receive an electronic page proof for review and approval, following which the manuscript is published on the journal website.

Following acceptance, Authors are also requested to return both completed and signed Journal Publishing Agreement and Conflict of interest disclosure forms which they will be send with the proof.

Full Authors Guidelines and online Submission System link are available on Journal website: http://www.eurannallergyimm.com/.

Manuscripts should be typewritten (double spacing) on one side of the paper; on a separate sheet (Title page) should bear the title of the paper, short title, authors, affiliations, complete address and e-mail of the corresponding author, together with the name of institution where the work was done. To develop the study, the Authors should follow the rules according to the article type:

- **Original article**
- **Review (by invitation only)**
- **Opinion paper**
- **Letter to the Editor**
- **Commentary/Correspondence**

Each article must state:

- **Fundings**
- **Contributions of each author**
- **Conflict of interests**

References

References should respect:

- the order number corresponding with that of appearance in the text;
- the author's surname(s), followed by initial of first names;
- the title of the work, in the original language;
- for journals: usual title abbreviations according to international nomenclature and in this order: year;volume number(issue number):first and last page numbers of the work. doi.

For example:
Vaghi A, Bilò MB, Bini F, Cecchi L, Micheletto C, Musarra A. The added value of targeting airway hyperresponsiveness by blocking thymic stromal lymphopoietin (TSLP) in the management of severe asthma. Eur Ann Allergy Clin Immunol. 2025;(6):243-59. doi: 10.23822/EurAnnACI.1764-1489.376.

- For books: name of the author/editor, title, publisher/institution, town where published, year of publication, first and last page numbers of the work.

For example:
Paupe J, Scheinman P (eds.). Allergologie Pédiatrique. Flammarion, Paris, 1988: pp. 324-42.

Tables and illustrations

- Tables: always saved on separate numbered sheets;
- Figures: always saved as separate files (no .docx).
- Captions and legends: at the end of the text.
- Figures, diagrams: JPG, 300 dpi minimum.
- Radiographs: JPG, 300 dpi minimum.

All tables, figures, radiographs, etc. must be referenced in the text.

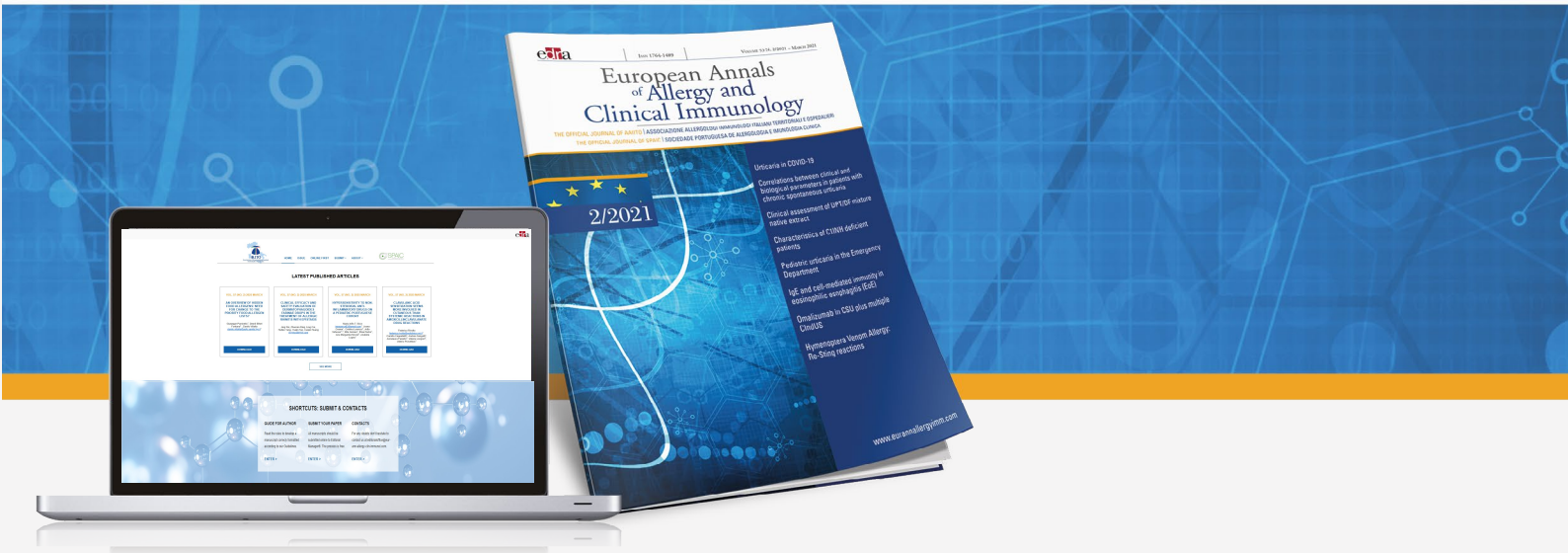
The "pdf" of the article will be sent to the author by e-mail.

Edra Media S.r.l.
Viale Enrico Forlanini 21
20134 Milan, Italy

THE OFFICIAL JOURNAL OF AAIITO
ASSOCIAZIONE ALLERGOLOGI
IMMUNOLOGI ITALIANI
TERRITORIALI E OSPEDALIERI

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF SPAIC
SOCIEDADE PORTUGUESA
DE ALERGOLOGIA
E IMUNOLOGIA CLINICA



- 6 print issues per year
- full access to www.eurannallergyimm.com, featuring all current and archived issues



European Annals of Allergy and Clinical Immunology

is a bimonthly peer-reviewed publication

- The official Journal of the "Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri" (Italian Association of Hospital Allergists and Immunologists - AAIITO) and the "Sociedade Portuguesa de Alergologia e Immunologia Clinica" (Portuguese Society of Allergology and Clinical Immunology - SPAIC)
- indexed in PubMed and Scopus
- collects reviews, original works concerning etiology, diagnosis and treatment of allergic and immunological disorders

16045

To submit your paper go to
https://www.editorialmanager.com/eaaci/default2.aspx

Edra Media S.r.l., Viale Enrico Forlanini 21, Milan