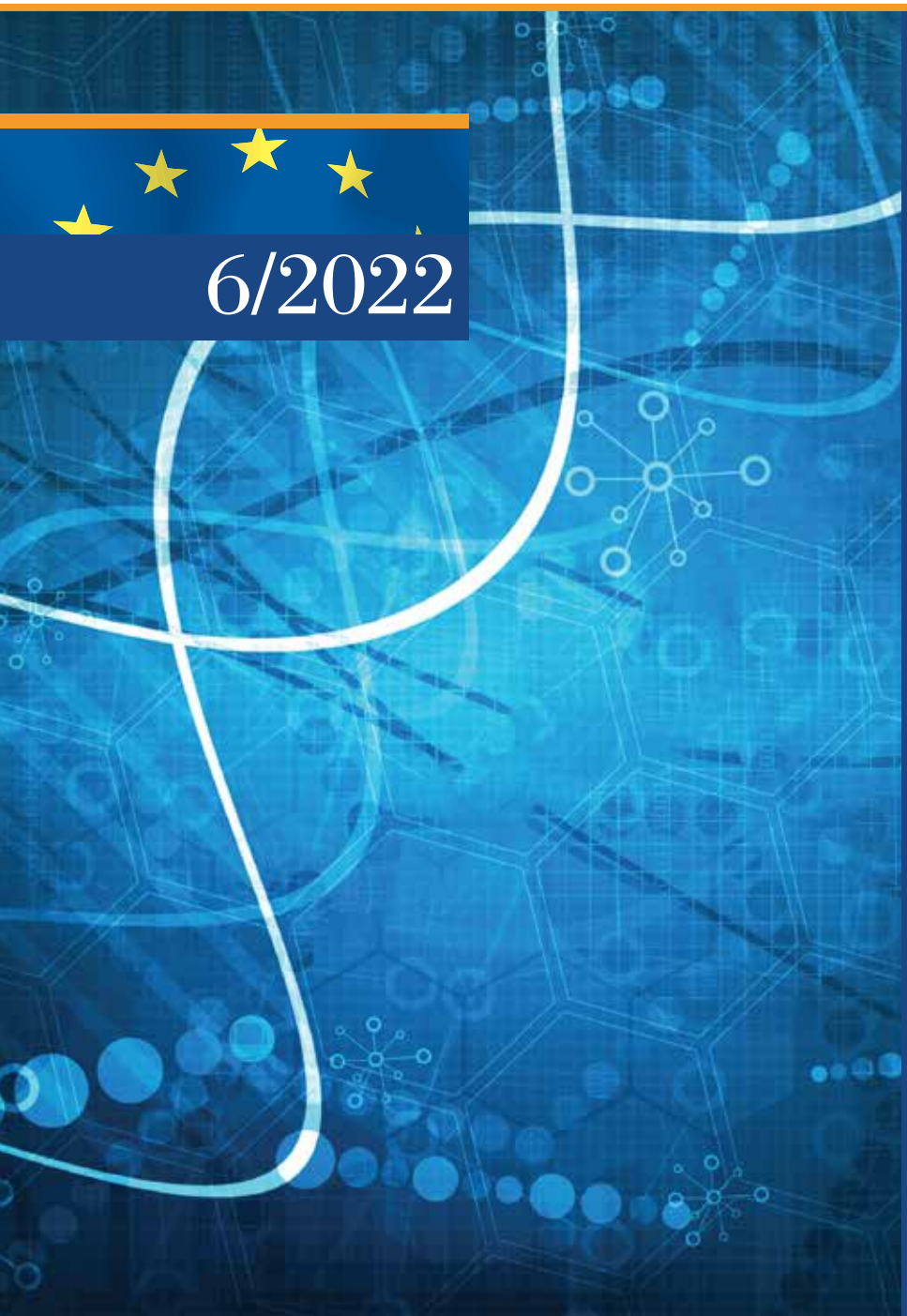
edra | Issn 1764-1489 | Volume 54 N. 6/2022 – November 2022

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



6/2022

www.eurannallergyimm.com

European Annals of Allergy and Clinical Immunology

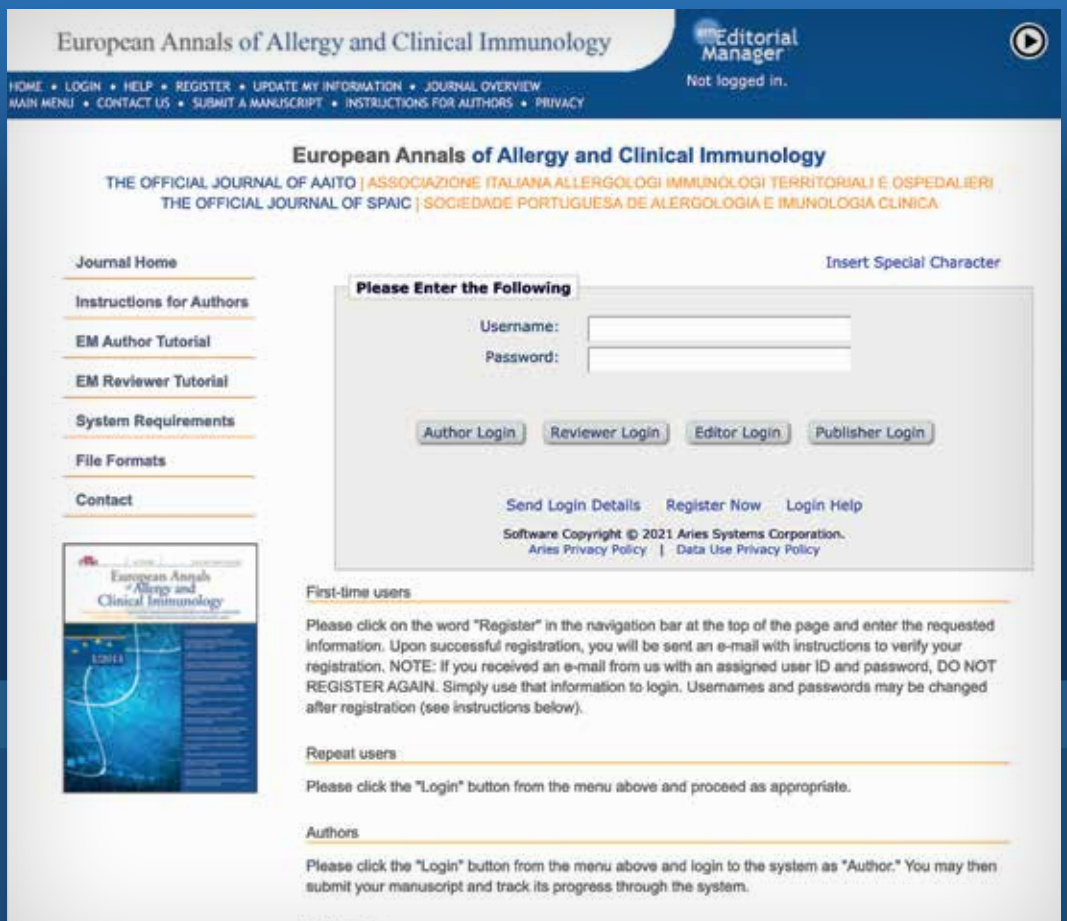
The online submission system

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

L. Cecchi (Italy)
P. Carreiro-Martins (Portugal)

DEPUTY EDITORS

R. Rodrigues Alves (Portugal)
M.B. Bilò (Italy)

ASSOCIATE EDITORS

R. Asero (Italy)
M. Branco Ferreira (Portugal)
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

Editors in Chief and Managing Directors

Lorenzo Cecchi
P. Carreiro-Martins

Chief Business & Content Officer

Ludovico Baldessin

Editorial Coordinator

Barbara Moret

Publishing Editor

Jessica Guenzi
j.guenzi@lswr.it
Ph. 039 (0)2-89293.926

Production Manager

Paolo Ficicchia
p.ficicchia@lswr.it
Ph. 0039 (0)2-88184.222

Sales

Stefano Busconi
dircom@lswr.it
Ph. 0039 (0)2-88184.404

Subscription

abbonamentiedra@lswr.it
Ph. 0039 (0)2-88184.317
Italy subscription: 60 euro
World subscription: 85 euro

Printing

Rotomail Italia S.p.A., Strada Rivoltana (SP 14), 12/AB 20060 Vignate (MI), Italy

EDRA SpA

Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edizioniedra.it

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

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

To read our Privacy Policy please visit www.edraspa.it/privacy

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
Riccardo Asero

Designated President
Lorenzo Cecchi

Vice President
Danilo Raffaele Villalta

Treasurer
Oliviero Quercia

Past President
Antonino Musarra

Members
Lucio Bonazza
Paolo Borrelli
Gabriele Cortellini
Battista Roberto Polillo
Valerio Pravettoni
Giuseppe Valenti
Maria Teresa Zedda

SPAIC
Sociedade Portuguesa de Alergologia e Imunologia Clínica

Directory Board

President
Manuel Branco-Ferreira

Past President
Elisa Pedro

Vice Presidents
Ana Morete
José Ferreira
Pedro Martins

Treasurer
Rodrigo Rodrigues Alves

Secretary-General
Emilia Faria

Secretary-Adjunct
Frederico Regateiro

Members
João Fonseca
Ângela Gaspar
Natacha Santos

Table of Contents

L. Cecchi[1,2], A. Vaghi[3], F. Bini[4], M. Martini[5,6], A. Musarra[7], M. B. Bilò[5,8]

From triggers to asthma: a narrative review on epithelium dysfunction

[1]SOS Allergy and Clinical Immunology, USL Toscana Centro, Prato, Italy
[2]Centre of Bioclimatology, University of Florence, Florence, Italy
[3]Former Head of Pneumology and Chief of Department of Medicine and Rehabilitation, Guido Salvini Hospital-ASST-Rhodense, Garbagnate Milanese, Milan, Italy
[4]UOC Pneumology, ASST-Rhodense, Garbagnate Milanese, Milan, Italy
[5]DISCLIMO - Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Italy
[6]Allergy Unit, Ospedali Riuniti Marche Nord, Fano, Italy
[7]Allergy Unit, National Healthcare System, Scilla, Italy
[8]Allergy Unit, Department of Internal Medicine, University Hospital Ospedali Riuniti di Ancona, Ancona, Italy

Key words

Epithelial cytokines; airway epithelium; environment; barrier; allergy.

Corresponding author
Lorenzo Cecchi
SOS Allergy and Clinical Immunology
USL Toscana Centro
Piazza Ospedale 1
59100 Prato, Italy
ORCID ID: 0000-0002-0658-2449
E-mail: lorenzo.cecchi@unifi.it

Doi
10.23822/EurAnnACI.1764-1489.271

Summary

It is currently recognized that the airway epithelium plays a pivotal role in orchestrating inflammatory, immune, and regenerative responses to allergens, viruses and environmental pollutants that contribute to asthma pathogenesis. The impact of pollen on respiratory epithelium is multifaceted and goes beyond the direct barrier damage driven by the best-known Type-2 response. After pollen-driven activation, airway epithelial cells play an active role in triggering several pathways. In particular, the release of epithelial cytokines (or alarmins) activates both innate and adaptive immunity, with downstream effects implicated to the pathogenesis of asthma. Pollutants also have a pleiotropic effect on respiratory epithelium. Diesel exhaust particles can directly damage the respiratory epithelium with consequent barrier dysfunction, increased permeability, and local inflammation, but they can also activate Th2 responses. Innate immune responses also are triggered by pollutants through release of epithelial cytokines and redox-sensitive pathways that generate mechanical and immunologic changes in the respiratory epithelium. In addition to the typical Type-1 immune response, respiratory virus infections stimulate type-2 innate lymphoid cells in the airway epithelium to release epithelial cytokines. Finally, the action of epithelial triggers on airway smooth muscle is the central element in the induction of remodeling and hyperreactivity of the airways in asthma. This article reviews the pathophysiology and functions of the airway epithelium and the role of epithelial damage by different triggers in the development, persistence, and exacerbations of asthma.

Introduction

Asthma is the most common respiratory disease, reported to affect up to 18% of the population depending on the country for a total of over 300 million patients worldwide. Most patients have mild disease; however, over 20% have difficult-to-treat or severe asthma (1) that remains uncontrolled despite standard-of-care therapy. Chronic airway inflammation, airway hyper-responsiveness to inhaled triggers, and airway remodeling are pathophysiological pillars of asthma (2).
Asthma airway inflammation is characterized by a multicellular process involving mainly eosinophils, neutrophils, $CD4^+$ T lymphocytes, monocytes, mast cells, and basophils (3). Upregulation of different cell types and biomarkers configures different inflammatory phenotypes, the most prevalent is the eosinophilic type 2 (T2) inflammatory phenotype, wherein a high number of eosinophils are present in sputum, airway, and/or blood, while in the minority non-eosinophilic phenotype the dominant inflammatory cell types may include neutrophils, mixed granulocyte inflammatory cells, or very few inflammatory cells (so-called paucigranulocytic inflammation) (4). Both innate and adaptive immune responses are involved in the inflammatory responses in asthma, with T-lymphocyte immunity and $CD4^+$ Th2 cells playing a crucial role (3). Enhanced

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

immune and inflammatory responses in asthma are associated with structural changes (remodeling) in all elements of the airway wall, which is another major pathological feature of asthma, as important as inflammation in the pathogenesis of the disease and linked with inflammation by a bidirectional interaction. Airway remodeling in asthma implies cellular and extracellular matrix changes in the large and small airways, epithelial cell apoptosis, airway smooth muscle cell proliferation, and fibroblast activation, mediated by crosstalk of different cell types within the airway wall and submucosa. Three integrated and dynamic processes are involved in airway remodeling: initiation by epithelial cells; amplification by immune cells; and mesenchymal effector functions (5).

The airway epithelium represents a first-line physical, chemical, and immunological defence against the penetration of inhaled potentially toxic or damaging environmental insults. In the last decade, evidence has grown that alterations in the physical and functional barrier properties of the bronchial epithelium play a role that is no less than allergic pathways in the origin and clinical manifestations of asthma. Continued epithelial exposure to viral, allergenic, and polluting triggers along with a progressively reduced reparative response create the conditions for the persistence of inflammation, remodeling of the airway wall and subsequently persistence of asthma symptoms (6, 7). Therefore, targeting inflammation alone may not be sufficient to provide optimal clinical benefits. Here we review the pathophysiology and functions of the airway epithelium and the role of epithelial damage by different triggers in the development, persistence, and exacerbations of asthma.

The role of airway epithelium in asthma

The airway epithelium represents the first barrier to environmental stressors – air pollutants, microbial pathogens, and allergens – and plays a major role in their neutralization by its muco-ciliary clearance (MCC). In addition to these barrier and cleansing functions, there is evidence that the airway epithelial cells (AECs) are involved in the inflammatory response to damage from inhaled agents and exert immunological functions by interacting with the cells of the immune system (8). The loss of the airway epithelial barrier function in asthma is a consequence of the interaction between environmental factors (exposome), genetics and epigenetic regulatory mechanisms. Three types of intercellular epithelial junctions contribute to the barrier role of the airway epithelium, by linking the intracellular structures of one epithelial cell to the next: adherent junctions (AJs), hemidesmosomes, and tight junctions (TJs). AJs interconnect the actin filaments of the adherent cells; hemidesmosomes form adhesive bonds between the cytoskeleton of epithelial cells and the *lamina lucida* of the *lamina propria*; TJs form a multiprotein junctional complex called *zonula occludens* (ZO) that regulates paracellular permeability (9). In healthy conditions, TJs and AJs form a dense protein network interconnecting epithelial cells, which prevents the passage of virtually all molecules, including pathogens or other inhaled particles (10). In asthma patients, there is strong evidence that disruption of the airway epithelium occurs, impairing its barrier function (11).

Four different factors are recognized to damage the integrity of the airway epithelial barrier in the pathogenesis of asthma, by disrupting epithelial cell junctions: aeroallergens, environmental pollutants, viral infections, and allergic inflammation. Genetic and epigenetic vulnerability of the epithelium in asthma patients favors greater damage by the exposome favoring a self-reinforcing circle. Following epithelial damage, mediators like thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 – called epithelial cytokines or alarmins – are rapidly released from epithelial cells, activating innate and adaptive responses in distinct, though overlapping, ways (12). Epithelial cytokines all regulate a broad spectrum of innate immune cell populations (**table I**) and are particularly potent in eliciting and activating type 2 innate lymphoid cells (ILC2s), involved throughout the allergic inflammation process (12). Receptors for epithelial cytokines are highly expressed by subpopulations of Th2 memory cells and this supports their role in allergic exacerbations. Furthermore, the TSLP/ILC axis was recently shown to mediate steroid resistance in asthma (12).

TSLP is a member of the IL-2 family and a regulator of T2 dependent and non-T2 dependent inflammatory responses. The main source of TSLP are epithelial cells, especially skin and lung epithelial cells (13), although other possible cellular sources include mast cells, dendritic cells (DCs), fibroblasts, and airway smooth muscle cells (12, 14, 15). TSLP has two isoforms: the short isoform is expressed constitutively during homeostasis and is important for anti-inflammatory, barrier integrity and anti-microbial responses, while the long isoform is expressed during inflammation and supports inflammatory cytokine production (16).

Genetic variations of TSLP are associated with an increased risk of developing asthma. It has been shown that both genetic mutations and continuous exposure to allergens can induce an overproduction of TSLP (17). Multiple clinical features of asthma are associated with TSLP expression: asthma severity (18), reduced lung function (18), airway remodeling (19), reduced steroid response (21), exaggerated T2 response to viral infections (22). TSLP plays a key role in driving allergic inflammation by (I) upregulating the expression of MHCII and co-stimulating molecules in DCs, thus facilitating antigen presentation by DCs to $CD4^+$ naive T cells, and (II) inducing the upregulation of the expression of the OX40 ligand on DCs, thus accelerating differentiation of $CD4^+$ naive T cells into Th2 cells (23, 24).

TSLP and IL-33 synergize in activating ILC2s stimulating them to produce IL-4 and IL-13, which contribute to the epithelial barrier dysfunction in asthma by suppressing the expression of TJs and AJs proteins (25).

It has been shown that TSLP may also promote the differentiation of naïve $CD4^+$ T cells in Th17 cells producing IL-17A

Table I *- Cellular targets and pathogenic effects of epithelial cytokines (or alarmins) in asthmatic airways.*

Cell type	Functional effect		
	IL-25	**IL-33**	**TSLP**
Monocytes/ macrophages/ alveolar macrophages	↓ Rab27a and Rab27b expression ↓ Release of exosomes	↑ M2 macrophage polarization ↓ ADAMTS family of metalloproteases Signaling through ERK1/2, JNK, and PI3k-Akt	↑ TARC/CCL17, PARC/CCL18, MDC/CCL22, MIP3 /CL19 ↑ CD80 ↑ M2 macrophages
Dendritic cells/ myeloid dendritic cells	↑ Activated Th2 memory cells ↑ Chemotaxis of IL-9 producing cells	↑ Th2 polarization ↑ $CD4^+$ T cell release of IL-5 and IL-13 ↑ Macrophage release of IL-13	↑ MHC class II, CD40, CD86, CD54, CD80, CD83 ↑ OX40L ↑ IL-8, eotaxin-2, TARC/CCL17, MDC/CCL22, I-309/CCL1 ↑ Expansion of $CRTH2^+$ $CD4^+$ Th2 memory cells ↑ Differentiation of Tregs Signals through Jagged-1, JAK1, JAK2, Akt, ERK, JNK, NF-kB (p50, RelB), STAT1, STAT3, STAT4, STAT5, STAT6
Mast cells	Receptor expressed but function not defined	↑ Mast cell survival, adhesion, cytokine production ↑ IL-6, IL-13 MK2/3 activation of ERK1/2, PI3k c-Kit activation of ERK1/2, JNK1, PKB, and STAT3	↑ IL-5, IL-13, IL-6, IL-10, IL-8, GM-CSF ↑ CXCL8, CCL1 ↑ TGF-β
Basophils	↓ Apoptosis ↑ Histamine degranulation, IL-4, IL-13	↑ Histamine, IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, MCP, MIP ↑ CD11b expression ↑ Adhesion and priming of eotaxin-induced migration Signaling through ERK1/2, JNK, p38, and NF-kB	↑ CD69, CD62L, CD11b, CD123, IL-33R, IL-18R surface expression ↑ IL-4, IL-13 ↑ CD203c, IL17RB expression
Eosinophils	Eosinophil expression of IL-25 receptor ↑ MCP-1, MIP-1a, IL-8, IL-6, ICAM-1 ↓ ICAM-3, L-selectin Signaling through JNK, MAPK (p38), NF-kB	↑ Eosinophil survival, adhesion, degranulation ↑ Mature eosinophils and eosinophil progenitors from bone marrow ↑ Adhesion and survival ↑ Expression of CD11b ↑ Il-8 Signaling through MAPK (p38) and NF-kB	↑ Survival, adhesion ↑ CD18, ICAM-1, CXCL8, CXCL1, CCL2, IL-6 ↓ L-selectin Signals through ERK, p38, NF-kB
ILC2	↑ IL-4, IL-5, IL-13 ↑ Expression of IL-33R Signaling through MAPK	↑ IL-5, IL-13 Signaling through PI3k/AKT/mTOR, MAPK (p38)	↑ IL-25R, IL-33R expression
Natural killer T cells	↑ IL-13 ↑ CCL17, CCL22, C10/ CCL6, ECF-L	↑ IL-4, IFN-γ	↑ IL-4, IL-13

Cell type	Functional effect		
	IL-25	**IL-33**	**TSLP**
$CD4^+$ T cells	↑ IL-4, IL-5, IL-13 ↑ CD3, CD8 cells	↑ IL-9	↑ Proliferation ↑ Differentiation Signals through STAT1, STAT5, JAK1, JAK2
$CD34^+$ progenitor cells			↑ Eosinophilopoiesis and basophilopoiesis ↑ IL-5, IL-13, GM-CSF, CCL22, CCl17, CXCL8, CCL1 ↑ IL-5Rα expression
$CD8^+$ T cells			↑ Proliferation Signals through STAT5, Bcl-2
T regulatory cells			↓ Development ↑ Differentiation ↓ IL-10
Th2 cells	↑ IL-4, IL-5, IL-13 Signaling through STAT5	↑ IL-4, IL-5, IL-13 ↓ IL-4, IL-5, IL-13 in certain conditions Signaling through PI3k/AKT/mTOR and MAPK	↑ Proliferation ↑ Differentiation ↑ IL-5, IL-4, IL-13
Th9 cells	↑ Inhibit Th2 differentiation		
B cells			↑ Proliferation ↑ Development Signals through STAT1, STAT3, STAT5, JAK1, JAK2
Epithelial/ endothelial cells	Receptor expression ↑ Angiogenesis ↑ Endothelial cell VEGF/ VEGF receptor expression) Signaling through PI3K/Akt and Erk/MAPK	↑ IL-8 Signaling through ERK and MAPK (p38)	↑ Airway obstruction mechanisms Signals through TARC/CCL17, MDC/CCL22, IP-10/CXCL10
Airway smooth muscle	↑ TNF-α ↓ INF-y ↑ EMC procollagen-aI and lumican mRNA Signaling through NF-kB		↑ IL-6, CXCL8, CCL11 ↑ Migration, actin polymerization, cell polarization Signals through STAT3, MAPKs (ERK1/2, p38 and JNK)
Fibroblasts	↑ CCL5, CCL11, GM-CSF, CXCL8		

ADAMTS: A Disintegrin and Metalloproteinase with Thrombospondin motifs; AKT: protein-kinase B; Bcl-2: B-cell lymphoma 2; CCL: C-C motif chemokine ligand; CRTH2: Chemoattractant receptor-homologous molecule expressed on TH2 cells; CXCL: C-X-C motif chemokine ligand; ECF-L: Eosinophil chemotactic factor L; EMC: Extracellular matrix components; ERK: extracellular signal-regulated kinase; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN-γ: Interferon-γ; ICAM: intercellular adhesion molecule; IL: interleukin; IP-10: Interferon-γ inducible 0rotein 10; JAK: Janus kinase; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; MCP: monocyte chemoattractant protein; MDC: macrophage-derived chemokine; MIP: macrophage inflammatory protein; MHC: major histocompatibility complex; MK: MAPK-activated protein kinases; mTOR: Mammalian target of rapamycin; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; PARC: pulmonary and activation-regulated chemokine; PK: protein-kinase; PI3k: phosphoinositide 3-kinase; STAT: signal transducer and activator of transcription; TARC: thymus- and activation-regulated chemokine; TNF: Tumor necrosis factor; TSLP: thymic stromal lymphopoietin; VEGF: Vascular endothelial growth factor. Modified from Whetstone CE *et al.* (29).

(26), whose various effects in asthma pathophysiology have been demonstrated, including stimulation of bronchial epithelial cells to produce neutrophilic-promoting cytokines such as IL-8 and GM-CSF and promotion of airway remodeling by altering smooth muscle cell function, as detailed below (27). The effects of TSLP in airway remodeling also include stimulation of human fibroblasts, which express the TSLP receptors, to significantly increase collagen and alpha actin production (28).

To complete the overview on the role of epithelial cytokines, it is worth remembering that IL-25, a member of the IL-17 cytokine family, is expressed in airway epithelium as a preformed cytokine and stored in the cytoplasm, ready to be rapidly released following cell stimulation by environmental triggers, including allergens. IL-25 directly enhances Th2 cytokine production from Th2 memory cells activated by TSLP. IL-25 release by airway epithelial cells contributes to many pathogenic features of asthma, including the recruitment of eosinophils, airway mucus over secretion, and airway remodeling (29).

IL-33 is one of the earliest cytokines released in response to allergens and is central in the activation of both the innate and adaptive immune response (30). IL-33 has been shown to be responsible for inducing early immune development and polarization toward type 2 T cell inflammation through two mechanisms: activating the maturation of resident dendritic DC and inducing DC-stimulated differentiation of naïve $CD4^+$ T cells into polarized Th2 cells (29). IL-33 levels are elevated in the lung epithelium, airway smooth muscle, and bronchoalveolar lavage, correlating with disease severity. In the lower airways, the release of IL-33 seems to be responsible for the development and exacerbation of airway hypersensitivity and asthma (29).

More recently, it has been shown that IL-5 can also participate in the reduction of contact between epithelial cells (31). Stimulation of IL-13 redirects the differentiation process of basal epithelial cells to produce more MUC5AC-positive cells and fewer ciliated cells and inhibits ciliogenesis while promoting cilia loss (32). Furthermore, during allergenic stimulation, following the production of IL-13, the club cells (Clara cells) become mucus-producing cells due to a metaplastic and non-proliferative process (33). Overall, the cells reprogrammed by IL-13 produce a mucus with modified characteristics that has lost its innate immunity-related characteristics. This modified mucus slows the rate of the ciliary beat and stops muco-ciliary transport (34). In fact, increase in type 2 inflammation has been shown to be associated with decreasing MCC, although in mild inflammation, high rates of MCC can be found, indicating a compensatory mechanism, which is lost with high levels of inflammation (35). Whether such impairment in MCC can lead to worse clinical outcomes in severe asthma needs further studies.

All epithelial cytokines act upstream of T2 inflammation and at least in part also of non-T2 disease. In conclusion, the bronchial epithelium has an important gatekeeping function, and its dysfunction can affect both the induction and the progression of asthma.

The epithelial response to the aeroallergen trigger: the example of pollen

In addition to the well-known involvement of the adaptive immune system, the innate immune system seems to play a key role in the pathogenesis of asthma. In fact, allergens may trigger early warning signals through the activation of cells of the innate immune system. Therefore, according to the epithelial barrier hypothesis, allergens not only may induce a type 2 immune response but are involved in the early pathogenesis of asthma from the first contact with respiratory tissues (36). In response to this first contact, AECs promote both the activation of the innate immune system, with the production of cytokines and danger signals, and type 2 immunity, by activating DCs. This might be the first step in the pathogenesis of asthma, before the activation of the adaptive immune system and the type 2 damage mechanism. The airway epithelium should be therefore considered not only the first target of external triggers, but also the first active effector, acting as a bridge between the innate and the adaptive immune systems and playing a key role in activating the cascade of immunologic responses underlying allergen sensitization, asthma exacerbations and progression (37-39).

Phase 1 – Entry of aeroallergens into the airways

As reviewed above, anatomical and functional barriers prevent the contact between allergens and airway tissues. However, allergens may overcome these physical barriers due to physical and functional changes associated with asthma, such as loss of epithelial integrity, impaired ciliary function, reduced mucus clearance caused by increased mucus viscosity and swelling. On the other hand, climate change and global warming were shown to increase allergen concentration and allergenicity (*e.g.*, duration of pollination, amount of released allergens), and the occasions of exposure (*e.g.*, thunderstorms), with negative effects on respiratory health and increased risk of asthma (38).

Phase 2 – Allergen interaction with airway tissues

Allergens can interact through various mechanisms with the airway tissues, especially the epithelium as first contact. First, the direct proteolytic activity of some allergens (*e.g.*, cysteine proteinase of Der p 1) can lead to disruption of the airway epithelial barrier through cleavage of molecules in the tight junctions (occluding, claudin), possibly enhanced by genetic predisposition in asthmatic subjects, and to the loss of the apicobasal polarity of AECs (40). Consequently, the increased permeability to airways DCs favors the subsequent Type 2 activation pathway of allergen sensitization, and apical cytokine receptors (*i.e.*, normally expressed on the apical side of AECs) have access to basolateral cytokines. Another possible mechanism by which allergens interact with airway tissues is their binding with pattern recognition receptors (PRRs), protease-activated receptors (PARs), and toll-like receptors (TLR) of AECs, which triggers the downstream cascade of both innate and adaptive immunity.

In asthmatic subjects, genetic predisposition, epigenetic modifications from previous/chronic allergen exposure (*i.e.*, immunological imprinting), or both may facilitate this activation and dictate the type of consequent response (*e.g.*, Type 2 or Type 1 polarization) (41).

Phase 3 – Airway epithelium activation: more than just a passive barrier

The airway epithelium can actively react after the interaction with allergens. Therefore, the easier access of allergens to the underlying tissues is not only the effect of the epithelial damage, but of several other mechanisms triggered by the epithelial activation, with effects on both innate and adaptive immune systems. Damage-associated molecular patterns (DAMPs) are molecules released from injured AECs, able to activate pathways regulated by NFkB, MAP-kinases, and interferon regulatory factors (IRFs) (42). Epithelial cell-derived cytokines are released by AECs in case of stress or death (IL-25, IL-33, HM-GB1, uric acid, ATP). Higher levels of epithelial cytokines have been found in subjects with allergic asthma, compared with healthy subjects, and genetic polymorphisms in genes coding for these types of cytokines and their receptors may justify these differences (37). DAMPs, epithelial cytokines, reactive oxygen species (ROS), and other inflammatory mediators acting as danger signals promote the early recruitment of innate immune cells like ILC2s, basophils, macrophages, and DCs and contribute to the Th2 polarization of the adaptive immune system. In addition, they are also responsible for morphological and functional changes of the airways, possibly inducing goblet cell metaplasia and change in mucus characteristics (43), with detrimental effects on the anatomical barriers against allergen entrance (37-39).

The interactions between aeroallergens and the airway epithelium are depicted in **figure 1**.

The epithelial response to environmental pollutants: the example of diesel exhaust

Exposure to environmental pollutants has been associated with the development and exacerbation of asthma (44, 45). Diesel exhaust (DE) is a main contributor to air pollutants, capable to trigger Th2 immune responses which are directly associated with developing and aggravating allergic asthma and other respiratory diseases (46).

A wide range of animal and human nasal models have shown the negative pleiotropic effects of DE in damaging the airway epithelial barrier and augmenting allergic inflammation (44, 47). In *in vitro* studies, fine particulate matter (PM) and DE particles were shown to degrade TJ proteins such as occludin, claudin-1, and ZO-1 and downregulating claudin-1 expression in human airway cells (48). More recently, human nasal epithelial cells exposed to nontoxic ultrafine PM showed epithelial barrier dysfunction, with increased paracellular permeability and downregulated TJ proteins (49). DE was shown to induce sensitization to neoallergens, which did not arise with exposure to the neoantigen alone in allergic subjects, thus suggesting its important role in exaggerating the sensitization to allergens (47). Diesel exhaust particles (DEPs) were shown to promote dendritic cell maturation, possibly acting as adjuvants during allergic sensitization (50). DEPs were shown to increase the recall of eosinophils in the nasal mucosa in response to nasal allergic stimulation, potentiate the development of an IgE mediated response to new antigens, and increase the local level of IgE (51). PM and DEP can induce TSLP and IL-17A production and contribute to the development or exacerbation of chronic respiratory diseases (52). Activation of redox-sensitive pathways seems to play a major a role in the mechanical and immunologic changes induced by air pollution and antioxidant systems may normalize these negative effects (52). Interestingly, a study in patients with mild-to-moderate asthma showed reductions in the forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) when briefly exposed to DEPs in high traffic streets; decrease of both FEV1 and FVC was significantly greater than that measured in patients who walked for a similar time in an area not exposed to traffic (53).

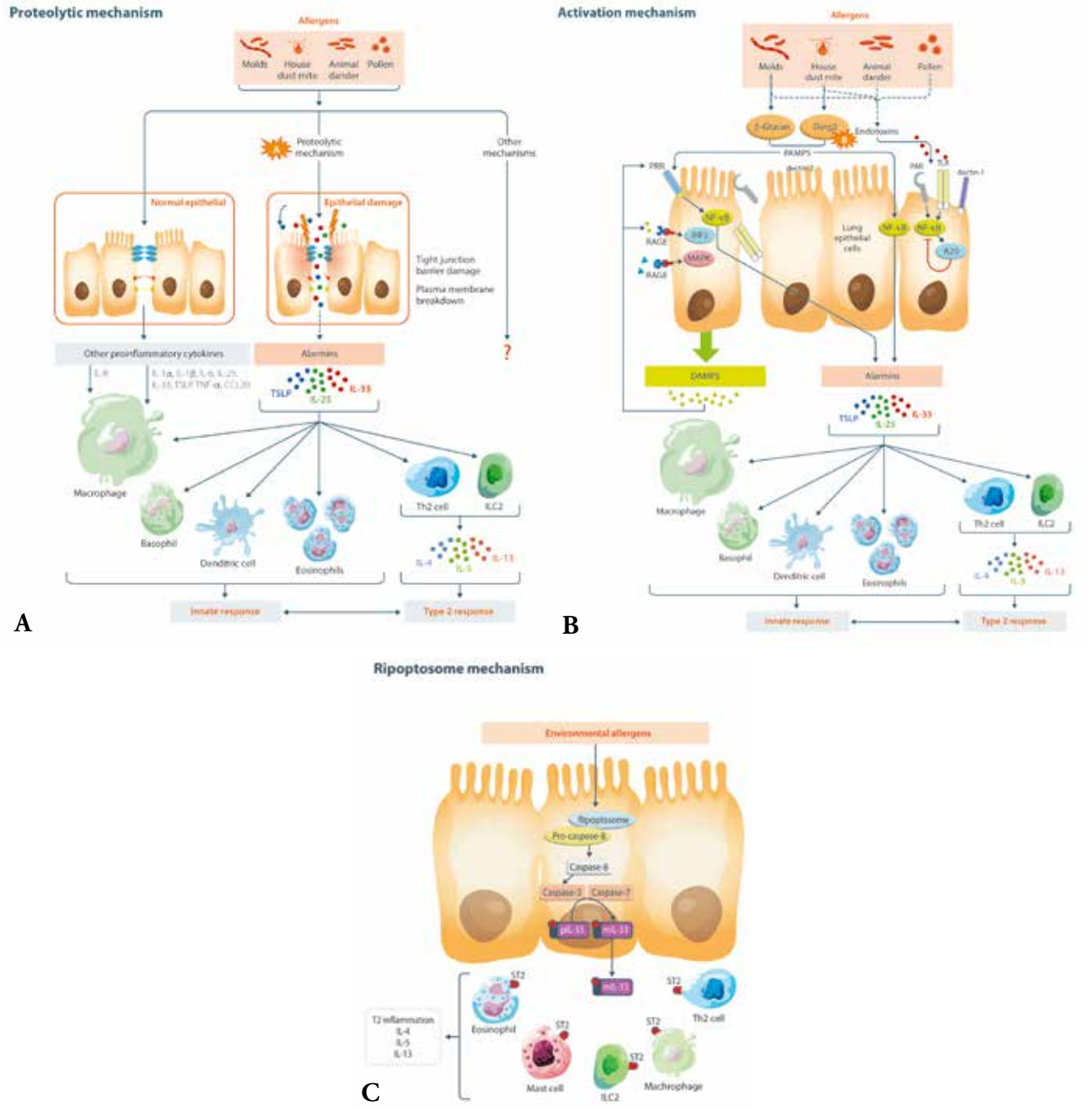
The interplay between air pollutants such as DEP and the immunopathogenesis of asthma is still object of intense research aimed at understanding how exposure to these agents can result in worsening of disease.

The epithelial response to infectious agents: the example of respiratory syncytial virus and rhinovirus

There is robust evidence that respiratory viruses, especially (RSV) and rhinovirus (RV), are associated with and may play a major role in the development and exacerbation of asthma. Early childhood infections with RV and RSV, the most common respiratory pathogens, have been associated with an increased risk of developing asthma later in life (54-57). Moreover, in subjects with asthma, respiratory viruses, particularly RV, can alter the host immune defence systems and trigger exacerbations in both children and adults (58, 59). Although the precise pathogenic mechanisms by which respiratory viruses may drive asthma development and exacerbations are not yet fully elucidated, great progress has been made in the last decade, suggesting that epithelial disruption by viruses and subsequent production of inflammatory and immune mediators may be the *primum movens* (43, 59).

Interferons (IFNs) are key components in the innate immune response of the airway epithelium to viral infection. For an effective antiviral response and viral clearance, interferon (IFN) production by epithelial cells is required. They exert their antiviral properties directly through the inhibition of viral replication in cells and indirectly through the stimulation of innate and adaptive immune responses. There is evidence that RV-induced epithelial IFN production is reduced and delayed in some individu-

Figure 1 *- Mechanisms of interaction between allergens and airway epithelium.*



Allergens of house dust mites (*e.g.,* Der p 1, Der f 1, Blo t 1, Eur m 1, Der m 1, Der p 3, Der p 6, Der p 9), cockroaches (*e.g.,* Per a 10), mould (*e.g., Aspergillus*, *Alternaria* species), animal dander, and pollens can interact with bronchial epithelial cells through proteolytic **(A)**, activation **(B)**, ripoptosome-mediated **(C)**, or other mechanisms. APC: Antigen presenting cell; CCL: C-C motif chemokine ligand; DAMPS: damage-associated molecular patterns; IL: interleukin; ILC2: type 2 innate lymphoid cells; IRF: Interferon regulatory factor; MAPK: mitogen-activated protein kinase; mIL: Mature interleukin; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; PAMPS: pathogen-associated molecular patterns; PAR: protease-activated receptors; pIL: Precursor of interleukin; PRR: pattern recognition receptor; RAGE: receptor for advanced glycation end-products; ST2: Suppressor of tumorigenicity 2; Th: T helper cell; TLR: toll-like receptor; TNF: Tumor necrosis factor; TSLP: Thymic stromal lymphopoietin.

als with asthma, and this may at least partly explain the increased susceptibility to viral infections of asthmatic patients (60).
Despite viral infection typically promotes a type 1 immune response, there is clear evidence indicating that it induces a type 2 inflammatory pattern as well, which is coordinated by the epithelium. It has been shown that airway epithelial cells from asthmatic individuals have an increased capacity to produce epithelial cytokines in response to virus infections, which may be responsible for exaggerated T2 inflammatory responses. Following viral infection, TSLP release from bronchial epithelial cells is increased in patients with asthma (61). When exposed to a viral surrogate, the epithelial cells from patients with asthma overexpress TSLP and underexpress IFN-β (62). In an experimental model of RV exacerbation, subjects with asthma had increased levels of IL-33, which correlated both with IL-5 and IL-13 levels in the airway lining fluid and with exacerbation severity following virus inoculation (63). In a similar model, experimental RV infection showed to induce higher IL-25 production and expression of IL-25 both at baseline and during infection in asthmatic individuals (64). Moreover, in response to viral infection, the airway epithelium directly stimulates ILC2s to release TSLP, IL-33, and IL-25, which in turn induce the release of IL-4, IL-5, and IL-13, major mediators of the type 2 inflammatory response (65, 66). In children with severe asthma increased level of ILC2s have been found (67).Viral infections not only initiate an immune response but also participate in remodeling the epithelial barrier and the subepithelial extracellular matrix (ECM). Kuo *et al*. produced evidence suggesting that viruses may contribute to airway remodeling through increased ECM deposition, which in turn may contribute to increased airway smooth muscle mass increasing cell migration (68, 69).
In conclusion, in response to viral infection the bronchial epithelial cells can release epithelial cytokines and mediators that strongly stimulate T2-associated cytokine production by ILC2s, thus promoting airway inflammation and hyperresponsiveness. Viral infections may also contribute to airway remodeling by increased ECM deposition. Respiratory viral infections in early childhood may play a role in increasing the patient's susceptibility to asthma and other obstructive lung diseases later in life.

Relationship between epithelium and smooth muscle cells

Airway smooth muscle (ASM) cells play a central role in the pathogenesis of asthma by controlling airway muscle tone, balancing the extent of contraction *vs* dilation in response to local or circulating factors, and are therefore recognized as the primary cell type responsible for bronchoconstriction and airway hyperreactivity. ASM cells are also involved in the inflammatory and airway remodeling processes that occur in asthma (70). Epithelial triggers can stimulate proliferation, hyperplasia, and hypertrophy of ASM cells, which contribute to induction and modulation of airway wall structural changes (71). Activated ASM cells produce several chemotactic mediators and express different adhesion molecules which attract and favour the infiltration of inflammatory cells, mainly mast cells and T-lymphocytes. The mast cell infiltration of the ASM layer, called mast cell myositis, is a specific feature of asthma and is observed in most asthma phenotypes.
A crucial role in the epithelial response to microbial, traumatic, or inflammatory injuries, is played by epithelium produced TSLP, which potently activates mast cells. Mast cell activation increases the production of a broad range of chemokines and cytokines, which all contribute to the hypertrophy, hyperplasia, and hyperreactivity of ASM (72). This crosstalk between mast cells and ASM cells contributes to the persistence of airway inflammation and hyperresponsiveness in asthma (73). Another mechanism by which TSLP promotes airway remodeling in asthma is stimulating fibroblast cells to produce collagen through activation of the signal transducer and activator of transcription 3 (STAT3) (28, 74).
There is growing evidence suggesting that the migration of ASM cells may also contribute to cellular hyperplasia, thus contributing to the increase of ASM mass. The source of these migrating cells is still not fully elucidated. The increase in ASM mass may be further due to airway infiltration of myofibroblasts, adjacent ASM cells, or circulating hemopoietic progenitor cells (75). TLSP-induced STAT-3 activation was shown to also exert a pro-migratory function, further supporting TLSP role in ASM remodeling (76).
ASM cells also produce and secrete exosomes, extracellular membranous nanovesicles implicated in intercellular communication, which have recently been shown to play a pivotal role in the pathology of asthma and other inflammatory diseases. Exosomes seem to influence and modify the functionality of inflammatory and structural lung cells, contributing to the characteristic processes of asthma disease (77). Neuropeptide Y (Npy), which has been reported to be ectopically expressed in the airways of asthma patients, was shown to induce ASM contraction. This suggests a role for paracrine signals from the airway epithelium to ASM to induce airway responsiveness (78).

Conclusions

Robust evidence indicates that the airway epithelium is dysfunctional in asthma, and plays a critical role in the development, progression, and exacerbation of the disease. Structural and functional anomalies of the airway epithelium result from the interaction between genetic, epigenetic, and environmental factors (exposome) and orchestrate the inflammatory response and bronchial remodeling. Aeroallergens and pollutants acting as epithelial triggers activate several pathways. In particular, the release of epithelial cytokines activates both innate and adaptive immunity, with downstream effects implicated to the pathogenesis of asthma. Further studies on their mechanism of action might help to elucidate their role also as a target for therapies that might be able

to treat respiratory diseases regardless of the specific pathogenetic mechanism downstream the release of epithelial cytokines.
The effect of epithelial triggers on ASM is the key factor in the induction of remodeling and hyperreactivity of the airways. Inhibition of TSLP and IL 13 might prevent both mast cells activation and collagen production by fibroblasts. There is a need of new therapeutic tools able of acting on extracellular vesicles and neuropeptides involved in the inflammatory process and in ASM cells contraction, and therefore on the hyperreactivity of the airways.
Another current priority is the search for biomarkers that can allow to identify the presence and possibly the severity of the damage and epithelial dysfunction. This new field of investigation may have important implications in detecting pathogenetic mechanisms and disease endotypes (T2-dependent and T2-non dependent), identifying subjects with a greater risk of evolving towards persistent and severe forms of asthma, and developing new epithelial-centred therapeutic strategies (*e.g.*, anti TSLP or anti IL-33).

Fundings

None.

Conflict of interests

Lorenzo Cecchi reports personal fees from Thermofisher, AstraZeneca, Sanofi Genzyme and Novartis outside the submitted work. Matteo Martini has none to declare. Antonino Musarra declares personal fees for Advisory Board work from AstraZeneca, GlaxoSmithKline and Sanofi Genzyme outside the submitted work. M. Beatrice Bilò reports personal fees from AstraZeneca, Novartis, GSK and Sanofi outside the submitted work. Adriano Vaghi reports personal fees from AstraZeneca, GSK and Sanofi. Francesco Bini declares that he has no conflict of interests.

Acknowledgements

Editorial and medical writing assistance was provided by EDRA S.p.A., respectively by Alessia Scotton (Editorial Project Manager, Edra S.p.A, Milan, Italy) and Renata Perego, PhD and funded by AstraZeneca.

References

1. 2022 GINA Report, Global Strategy for Asthma Management and Prevention. Available at: https://ginasthma.org/gina-reports/. Last access date: 09/18/2022.
2. Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, Casale TB, *et al*. A new perspective on concepts of asthma severity and control. Eur Respir J. 2008;32:545-54. doi: 10.1183/09031936.00155307.
3. Peebles RS Jr, Aronica MA. Proinflammatory Pathways in the Pathogenesis of Asthma. Clin Chest Med. 2019;40(1):29-50. doi: 10.1016/j.ccm.2018.10.014.
4. Carr TF, Zeki AA, Kraft M. Eosinophilic and non-eosinophilic asthma. Am J Respir Crit Care Med. 2018;197(1):22-37. doi: 10.1164/rccm.201611-2232PP.
5. Hough KP, Curtiss ML, Blain TJ, Liu RM, Trevor J, Deshane JS, Thannickal VJ. Airway Remodeling in Asthma. Front Med (Lausanne). 2020;7:191. doi: 10.3389/fmed.2020.00191.
6. Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev. 2011;242(1):205-19. doi: 10.1111/j.1600-065X.2011.01030.x.
7. Gohy S, Hupin C, Ladjemi MZ, Hox V, Pilette C. Key role of the epithelium in chronic upper airways diseases. Clin Exp Allergy. 2020;50(2):135-46. doi: 10.1111/cea.13539.
8. Frey A, Lunding LP, Ehlers JC, Weckmann M, Zissler UM, Wegmann M. More Than Just a Barrier: The Immune Functions of the Airway Epithelium in Asthma Pathogenesis. Front Immunol. 2020;11:761. doi: 10.3389/fimmu.2020.00761.
9. Buckley A, Turner JR. Cell biology of tight junction barrier regulation and mucosal disease. Cold Spring Harb Perspect Biol. 2018;10(1):a29314. doi: 10.1101/cshperspect.a029314.
10. Calvén J, Ax E, Rådinger M. The Airway Epithelium-A Central Player in Asthma Pathogenesis. Int J Mol Sci. 2020;21(23):8907. doi: 10.3390/ijms21238907.
11. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, *et al*. Defective epithelial barrier function in asthma. J Allergy Clin Immunol. 2011;128:549-56. doi: 10.1016/j.jaci.2011.05.038.
12. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. J Clin Invest. 2019;129:1441-51. doi: 10.1172/JCI124606.
13. Ziegler SF. Thymic stromal lymphopoietin and allergic disease. J Allergy Clin Immunol. 2012;130(4):845-52. doi: 10.1016/j.jaci.2012.07.010.
14. Bartemes KR, Kita H. Dynamic role of epithelium-derived cytokines in asthma. Clin Immunol 2012;143:222-35. doi: 10.1016/j.clim.2012.03.001.
15. West E, Kashyap M, Leonard WJ. TSLP: A Key Regulator of Asthma Pathogenesis. Drug Discov Today Dis Mech. 2012; 9(3-4):10.1016/j.ddmec.2012.09.003. doi: 10.1016/j.ddmec.2012.09.003.
16. Fornasa G, Tsilingiri K, Caprioli F, Botti F, Mapelli M, Meller S, *et al*. Dichotomy of short and long thymic stromal lymphopoietin isoforms in inflammatory disorders of the bowel and skin. J Allergy Clin Immunol. 2015;136(2):413-22. doi: 10.1016/j.jaci.2015.04.011.
17. He JQ, Hallstrand TS, Knight D, Chan-Yeung M, Sandford A, Tripp B, *et al*. A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness. J Allergy Clin Immunol. 2009;124(2):222-9. doi: 10.1016/j.jaci.2009.04.018.
18. Li Y, Wang W, Lv Z, Li Y, Chen Y, Huang K, Corrigan CJ, Ying S. Elevated Expression of IL-33 and TSLP in the Airways of Human Asthmatics In Vivo: A Potential Biomarker of Severe Refractory Disease. J Immunol. 2018;200(7):2253-62. doi: 10.4049/jimmunol.1701455.
19. Cao L, Liu F, Liu Y, Liu T, Wu J, Zhao J, *et al*. TSLP promotes asthmatic airway remodeling via p38-STAT3 signaling pathway in human lung fibroblast. Exp Lung Res. 2018;44(6):288-301. doi: 10.1080/01902148.2018.1536175.

20. Wu J, Liu F, Zhao J, Wei Y, Lv J, Dong F, *et al*. Thymic stromal lymphopoietin promotes asthmatic airway remodelling in human lung fibroblast cells through STAT3 signalling pathway. Cell Biochem Funct. 2013;31(6):496-503. doi: 10.1002/cbf.2926.
21. Liu S, Verma M, Michalec L, Liu W, Sripada A, Rollins D, *et al*. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. J Allergy Clin Immunol. 2018;141(1):257-68.e6. doi: 10.1016/j.jaci.2017.03.032.
22. Lee HC, Headley MB, Loo YM, Berlin A, Gale M Jr, Debley JS, *et al*. Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection. J Allergy Clin Immunol. 2012;130(5):1187-96.e5. doi: 10.1016/j.jaci.2012.07.031.
23. Murakami-Satsutani N, Ito T, Nakanishi T, Inagaki N, Tanaka A, Vien PT, *et al*. IL-33 promotes the induction and maintenance of Th2 immune responses by enhancing the function of OX40 ligand. Allergol Int. 2014;63:443-55. doi: 10.2332/allergolint.13-OA-0672.
24. Pelaia G, Vatrella A, Maselli R. The potential of biologics for the treatment of asthma. Nat Rev Drug Dis. 2012;11:958-72. doi: 10.1038/nrd3792.
25. Wise SK, Laury AM, Katz EH, Den Beste KA, Parkos CA, Nusrat A. Interleukin-4 and interleukin-13 compromise the sinonasal epithelial barrier and perturb intercellular junction protein expression. Int Forum Allergy Rhinol. 2014;4:361-70. doi: 10.1002/alr.21298.
26. Tanaka J, Watanabe N, Kido M, Saga K, Akamatsu T, Nishio A, *et al*. Human TSLP and TLR3 ligands promote differentiation of Th17 cells with a central memory phenotype under Th2-polarizing conditions. Clin Exp Allergy. 2009;39(1):89-100. doi: 10.1111/j.1365-2222.2008.03151.x.
27. Jones CE, Chan K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. Am J Respir Cell Mol Biol. 2002;26(6):748-53. doi: 10.1165/ajrcmb.26.6.4757.
28. Wu J, Liu F, Zhao J, Wei Y, Lv J, Dong F, *et al*. Thymic stromal lymphopoietin promotes asthmatic airway remodelling in human lung fibroblast cells through STAT3 signalling pathway. Cell Biochem Funct. 2013;31(6):496-503. doi: 10.1002/cbf.2926.
29. Whetstone CE, Ranjbar M, Omer H, Cusack RP, Gauvreau GM. The Role of Airway Epithelial Cell Alarmins in Asthma. Cells. 2022;11(7):1105. doi: 10.3390/cells11071105.
30. Yew Liew F, Girard J, Roderick Turnquist H. Interleukin-33 in health and disease. Nat Rev Immunol. 2016;16(11):676-89. doi: 10.1038/nri.2016.95.
31. Barretto KT. Human Airway Epithelial Cells Express a Functional IL-5 Receptor. Allergy. 2020;75(8):2127-30. doi: 10.1111/all.14297.
32. Tilley AE, Walters MS, Shaykhiev R, Crystal RG. Cilia dysfunction in lung disease. Annu Rev Physiol. 2015;77:379-406. doi: 10.1146/annurev-physiol-021014-071931.
33. Evans CM. Mucin is produced by Clara cells in the proximal airways of antigen-challenged mice. Am J Respir Cell Mol Biol. 2004;31:382-94. doi: 10.1165/rcmb.2004-0060OC.
34. Jackson N. Single-Cell and Population Transcriptomics Reveal Pan-epithelial Remodeling in Type2-High Asthma. Cell Rep. 2020;32(1):107872. doi: 10.1016/j.celrep.2020.107872.
35. Corcoran TE, Huber AS, Hill SL, Locke LW, Weber L, Muthukrishnan A, *et al*. Mucociliary Clearance Differs in Mild Asthma by Levels of Type 2 Inflammation. Chest. 2021;160(5):1604-13. doi: 10.1016/j.chest.2021.05.013.
36. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? Nat Rev Immunol. 2021;21:739-51. doi: 10.1038/s41577-021-00538-7.
37. Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. J Allergy Clin Immunol. 2014;134(3):499-507. doi: 10.1016/j.jaci.2014.06.036.
38. Celebi Sözener Z, Cevhertas L, Nadeau K, Akdis M, Akdis CA. Environmental factors in epithelial barrier dysfunction. J Allergy Clin Immunol. 2020;145(6):1517-28. doi: 10.1016/j.jaci.2020.04.024.
39. Frey A, Lunding LP, Ehlers JC, Weckmann M, Zissler UM, Wegmann M. More Than Just a Barrier: The Immune Functions of the Airway Epithelium in Asthma Pathogenesis. Front Immunol. 2020;11:761. doi: 10.3389/fimmu.2020.00761.
40. Carlier FM, de Fays C, Pilette C. Epithelial Barrier Dysfunction in Chronic Respiratory Diseases. Front Physiol. 2021;12:691227. doi: 10.3389/fphys.2021.691227.
41. Cayrol C. IL-33, an Alarmin of the IL-1 Family Involved in Allergic and Non Allergic Inflammation: Focus on the Mechanisms of Regulation of Its Activity. Cells. 2021;11(1):107. doi: 10.3390/cells11010107.
42. Patel S. Danger-Associated Molecular Patterns (DAMPs): the Derivatives and Triggers of Inflammation. Curr Allergy Asthma Rep. 2018;18(11):63. doi: 10.1007/s11882-018-0817-3.
43. Calvén J, Ax E, Rådinger M. The Airway Epithelium-A Central Player in Asthma Pathogenesis. Int J Mol Sci. 2020;21(23):8907. doi: 10.3390/ijms21238907.
44. Alexis NE, Carlsten C. Interplay of air pollution and asthma immunopathogenesis: a focused review of diesel exhaust and ozone. Int Immunopharmacol. 2014;23:347-55. doi: 10.1016/j.intimp.2014.08.009.
45. Zhang Q, Qiu Z, Chung KF, Huang SK. Link between environmental air pollution and allergic asthma: East meets West. J Thorac Dis. 2015;7:14-22. doi: 10.3978/j.issn.2072-1439.2014.12.07.
46. Ohtani T, Nakagawa S, Kurosawa M, Mizuashi M, Ozawa M, Aiba S. Cellular basis of the role of diesel exhaust particles in inducing Th2-dominant response. J Immunol. 2005;174(4):2412-9. doi: 10.4049/jimmunol.174.4.2412.
47. Nikasinovic L, Momas I, Just J. A review of experimental studies on diesel exhaust particles and nasal epithelium alterations. J Toxicol Environ Health B Crit Rev. 2004;7(2):81-104. doi: 10.1080/10937400490241952.
48. Caraballo JC, Yshii C, Westphal W, Moninger T, Comellas AP. Ambient particulate matter affects occludin distribution and increases alveolar transepithelial electrical conductance. Respirology. 2011;16:340-9. doi: 10.1111/j.1440-1843.2010.01910.x.
49. Zhao R, Guo Z, Zhang R, Deng C, Xu J, Dong W, *et al*. Nasal epithelial barrier disruption by particulate matter< 2.5 mm via tight junction protein degradation. J Appl Toxicol. 2018;38:678-87.
50. Bleck B, Tse DB, Curotto de Lafaille MA, Zhang F, Reibman J. Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation and polarization via thymic stromal lymphopoietin. J Clin Immunol. 2008;28:147-56. doi: 10.1007/s10875-007-9149-0.
51. Peden DB. The epidemiology and genetics of asthma risk associated with air pollution. J Allergy Clin Immunol. 2005;115(2):213-9. doi: 10.1016/j.jaci.2004.12.003.
52. Huff RD, Carlsten C, Hirota JA. An update on immunologic mechanisms in the respiratory mucosa in response to air pollutants.

J Allergy Cin Immunol. 2019;143:1989-2001. doi: 10.1016/j.jaci.2019.04.012.
53. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, Stewart-Evans J, Malliarou E, Jarup L, *et al*. Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med. 2007;357:2348-58. doi: 10.1056/NEJMoa071535.
54. Rossi GA, Colin AA. Respiratory syncytial virus-Host interaction in the pathogenesis of bronchiolitis and its impact on respiratory morbidity in later life. Pediatr Allergy Immunol. 2017;28:320-31. doi: 10.1111/pai.12716.
55. Régnier SA, Huels J. Association between respiratory syncytial virus hospitalizations in infants and respiratory sequelae: systematic review and meta-analysis. Pediatr Infect Dis J. 2013;32:820-6. doi: 10.1097/INF.0b013e31829061e8.
56. Rubner FJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, *et al*. Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence. J Allergy Clin Immunol. 2017;139(2):501-7. doi: 10.1016/j.jaci.2016.03.049.
57. Çalışkan M, Bochkov YA, Kreiner-Møller E, *et al*. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. N Engl J Med. 2013;368(15):1398-407. doi: 10.1056/NEJMoa1211592.
58. Johnston NW, Johnston SL, Duncan JM, Greene JM, Kebadze T, Keith PK, *et al*. The September epidemic of asthma exacerbations in children: a search for etiology. J Allergy Clin Immunol. 2005;115(1):132-8. doi: 10.1016/j.jaci.2004.09.025.
59. Rossi GA, Ballarini S, Salvati P, Sacco O, Colin AA. Alarmins and innate lymphoid cells 2 activation: A common pathogenetic link connecting respiratory syncytial virus bronchiolitis and later wheezing/asthma? Pediatr Allergy Immunol. 2022;33(6):e13803. doi: 10.1111/pai.13803.
60. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, *et al*. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med. 2005;201(6):937-47. doi: 10.1084/jem.20041901.
61. Lee HC, Headley MB, Loo YM, Berlin A, Gale M Jr, Debley JS, *et al*. Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection. J Allergy Clin Immunol. 2012;130(5):1187-96.e5. doi: 10.1016/j.jaci.2012.07.031.
62. Uller L, Leino M, Bedke N, Sammut D, Green B, Lau L, *et al*. Double-stranded RNA induces disproportionate expression of thymic stromal lymphopoietin versus interferon-beta in bronchial epithelial cells from donors with asthma. Thorax. 2010;65(7):626-32. doi: 10.1136/thx.2009.125930.
63. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, *et al*. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. Am J Respir Crit Care Med. 2014;190(12):1373-82. doi: 10.1164/rccm.201406-1039OC.
64. Beale J, Jayaraman A, Jackson DJ, Macintyre JDR, Edwards MR, Walton RP, *et al*. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. Sci Transl Med. 2014;6(256):256ra134. doi: 10.1126/scitranslmed.3009124.
65. Mjösberg J, Spits H. Human innate lymphoid cells. J Allergy Clin Immunol. 2016;138(5):1265-76. doi: 10.1016/j.jaci.2016.09.009.
66. Eberl G, Colonna M, Di Santo JP, McKenzie AN. Innate lymphoid cells: a new paradigm in immunology. Science. 2015;348:aaa6566. doi: 10.1126/science.aaa6566.
67. Nagakumar P, Puttur F, Gregory LG, Denney L, Fleming L, Bush A, *et al*. Pulmonary type-2 innate lymphoid cells in paediatric severe asthma: phenotype and response to steroids. Eur Respir J. 2019;54(2):1801809. doi: 10.1183/13993003.01809-2018.
68. Kuo C, Lim S, King NJ, Johnston SL, Burgess JK, Black JL, Oliver BG. Rhinovirus infection induces extracellular matrix protein deposition in asthmatic and nonasthmatic airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2011;300(6):L951-7. doi: 10.1152/ajplung.00411.2010.
69. Kuo C, Lim S, King NJ, Bartlett NW, Walton RP, Zhu J, *et al*. Rhinovirus induces expression of airway remodelling factors in vitro and in vivo. Respirology. 2011;16(2):367-77. doi: 10.1111/j.1440-1843.2010.01918.x.
70. Camoretti-Mercado B, Lockey RF. Airway smooth muscle pathophysiology in asthma. J Allergy Clin Immunol. 2021;147(6):1983-95. doi: 10.1016/j.jaci.2021.03.035.
71. Prakash YS. Airway smooth muscle in airway reactivity and remodeling: what have we learned? Am J Physiol Lung Cell Mol Physiol. 2013;305(12):L912–L933. doi: 10.1152/ajplung.00259.2013.
72. Kaur D, Doe C, Woodman L, Heidi Wan WY, Sutcliffe A, Hollins F, Brightling C. Mast cell-airway smooth muscle crosstalk: the role of thymic stromal lymphopoietin. Chest. 2012;142(1):76-85. doi: 10.1378/chest.11-1782.
73. Allakhverdi Z, Comeau MR, Jessup HK, Delespesse G. Thymic stromal lymphopoietin as a mediator of crosstalk between bronchial smooth muscles and mast cells. J Allergy Clin Immunol. 2009;123(4):958-60.e2. doi: 10.1016/j.jaci.2009.01.059.
74. Cao L, Liu F, Liu Y, Liu T, Wu J, Zhao J, *et al*. TSLP promotes asthmatic airway remodeling via p38-STAT3 signaling pathway in human lung fibroblast. Exp Lung Res. 2018;44(6):288-301. doi: 10.1080/01902148.2018.1536175.
75. Salter B, Pray C, Radford K, Martin JG, Nair P. Regulation of human airway smooth muscle cell migration and relevance to asthma. Respir Res. 2017;18(1):156. doi: 10.1186/s12931-017-0640-8.
76. Redhu NS, Shan L, Movassagh H, Gounni AS. Thymic stromal lymphopoietin induces migration in human airway smooth muscle cells. Sci Rep. 2013;3:2301. doi: 10.1038/srep02301.
77. Cañas JA, Sastre B, Rodrigo-Muñoz JM, Del Pozo V. Exosomes: A new approach to asthma pathology. Clin Chim Acta. 2019;495:139-47. doi: 10.1016/j.cca.2019.04.055.
78. Li S, Koziol-White C, Jude J, Jiang M, Zhao H, Cao G, *et al*. Epithelium-generated neuropeptide Y induces smooth muscle contraction to promote airway hyperresponsiveness. J Clin Invest. 2016;126(5):1978-82. doi: 10.1172/JCI81389.

R. Collado Chagoya, J. Hernández-Romero, A. A. Velasco-Medina, G. Velázquez-Sámano

Pilot study: specific immunotherapy in patients with Papular urticaria by *Cimex lectularius*

Department of Clinical Immunology and Allergy, Hospital General De México (Dr Eduardo Liceaga), Mexico City, Mexico

Key words

Specific immunotherapy; bed bugs; Cimex lectularius; papular urticaria; Prurigo Simplex.

Corresponding author
Rodrigo Collado Chagoya
Department of Clinical Immunology and Allergy
Hospital General De México (Dr Eduardo Liceaga)
Dr. Balmis 148, Doctores, Cuauhtémoc
06720 Ciudad de México, Mexico
ORCID ID: 0000-0002-9514-0297
E-mail: rodnova87@hotmail.com

Doi
10.23822/EurAnnACI.1764-1489.215

Impact statement

It is the first pilot study in Latin America on the usefulness of immunotherapy for the treatment of papular urticaria caused by bedbugs. Demonstrating effectiveness improving the number of lesions, quality of life and dermatological symptoms associated with the disease.

Summary

Background. *Papular urticaria is a chronic allergic reaction induced by insect bites. In México the most common causative arthropods reported are bed bugs, fleas and mosquitoes. Approximately 70% of people who are bitten by Cimex lectularius (C. lectularius) experience hypersensitive reactions, papular urticaria, extensive erythema, urticaria, and even anaphylaxis has been reported, Pruritus is the major complaint, impairing quality of life and sleep. Immunotherapy has been used in mosquito bite papular urticaria resulting in improvement of skin lesions and possibly protecting against reactions to subsequent exposures to mosquitoes.* ***Methods.*** *Children, 4-10 years of age, with recurrent papular urticaria due to bedbugs not responsive to multiple treatments were included. An initial allergy assessment included clinical history, skin prick test (SPT), and specific IgE sensitisation was performed to confirmed bedbug sensitization. Twenty children were randomized to receive subcutaneous specific immunotherapy (SSI) with whole body bed bug extract or conventional treatment. The treatment was carried out over twelve months and the response was assessed using the Dermatology Quality of Life Index (DLQ), the immunotherapy satisfaction questionnaire (ESPIA questionnaire) and the 12-Item Pruritus Severity Scale (12-IPSS). The results from both the treated and control groups were compared.* ***Results.*** *The twenty patients were randomized, 12 to receive immunotherapy and 8 to receive conventional treatment for 12 months. Quality of life improved with a reduction in the DLQI score of 19.83 in the immunotherapy group versus 9 in the conventional treatment group (p = 0.03). Itch improved with a reduction in the 12-IPSS of 16.5 in the immunotherapy group versus 9.63 in the conventional treatment group (p = 0.02). After twelve months of treatment, all 12 patients who received immunotherapy, reported a decrease of persistent cutaneous lesions but the 8 on conventional treatment did not. A mean score of 95.75 (SD 3.3) was recorded for satisfaction with immunotherapy.* ***Conclusions.*** *Patients with papular urticaria by C. lectularius receiving allergen immunotherapy for 1 year showed a significant improvement compared with baseline and patients receiving conventional treatment regarding skin lesions, quality of life impairment, intensity of pruritus and satisfaction with immunotherapy.*

Introduction

Papular urticaria also called lichen urticatus or prurigo simplex acuta (insect bites) is a chronic allergic reaction induced by insect bites, which is common in the tropics, in urban regions and in spring and summer. It is one of the most common dermatoses of childhood, with reported frequencies of 20% and 25% in Colombia and Venezuela respectively have been published (1, 2).
In Mexico the most common agents reported to cause papular urticaria are bed bugs, fleas and mosquitoes. Bed bugs are bloodsucking arthropod parasites of the *Hemiptera* order. Four genera are known: *Cimex, Leptocimex, Oeciacus* and *Haematasiphon* comprising 91 known species. Only three species cause bites in humans*: Cimex hemipterus, Cimex lectularius* and *Leptocimex boueti*. *Cimex lectularius* is most prevalent in temperate regions, whereas *Cimex hemipterus* is found mainly in tropical and subtropical regions and *Leptocimex boueti* predominates in Western Africa and South America (3). Bed bugs have been a persistent and scorned pest of humans, as referenced in recorded narratives dating back to classical Greek writings (Aristoteles

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

in the year 400 B.C.), medieval European texts and the Jewish Talmud. In London, in 1930, one-third of the population (approximately 4 million people) was estimated to be affected. The introduction of modern insecticides such as the organochlorine dichloro-diphenyl trichloroethane (DDT) provided a fast and an inexpensive method to control insect pests, including bed bugs (3). Unfortunately, bed bug infestations have rapidly increased worldwide over the last 20 years (4). Various factors have been postulated to be responsible for this reappearance. Overcrowded cities, a greater reliance on communal laundries, unregulated sale of second-hand clothing, use of previously owned furniture and furnishings, lack of family health care, a worldwide increase in secondary hosts including rodents, poultry, dogs and cats, lack of knowledge about the disease from the patient and from the health provider, an increase in local and international travel and migration, high costs of extermination processes coupled with insecticide resistance and toxicity of some others (5-9).

A variety of clinical reactions to bed bugs have been reported. Approximately 70% of the victims of *C. lectularius* bites will develop a cutaneous reaction and rarely a systemic reaction. These allergic reactions can vary from itchiness, an erythematous rash, urticaria, asthma and in the worst-case scenario, anaphylaxis. Pruritus is usually the cause of the impaired quality of life and sleep disturbances (10, 11). Cutaneous lesions usually start as small red macules that evolve to very pruritic wheals that last for several days causing the patient to enter an itch-scratch-itch cycle, which may lead to secondary bacterial infections. The lesions characteristically appear in exposed areas of the skin, such as the face, neck, hands and arms. The bites and pruritic papules display patterns that help identify the offending agent; appearing in pairs (dumbbells), following a linear or grouped triangular pattern with the lesions separated by a few millimeters, known as the "breakfast, lunch, and dinner" pattern. The bite itself is painless. Dependent on prior exposure, bites become symptomatic within minutes in those with prior sensitization or symptoms are delayed until sensitization has occued in first time exposed individuals. Lesions occur in crops in sensitized individuals with new local reactions developing while the old lesions heal. The most frequent complications of papular urticaria include ecthyma, cellulitis, cutaneous hyperpigmentation lymphangitis and impetigo. The reactions are sometimes complicated by insomnia and psycho-affective conditions such as anxiety, depression and psychotic states (12-14).

The diagnosis and identification of the responsible biting insect is clinical. *In vivo* analyses with skin prick test using the *C. lectularius* salivary gland solution can be used to confirm sensitization to *Cimex* in difficult cases. potential protein antigens present in the saliva of *C. lectularius* (15, 16).

Specific treatment involves removal from exposure and eradication of the insects which may prove to be difficult. Management should be based on education of the patient, improvements in personal hygiene, environmental hygiene and home hygiene and medical measures. Firstly, the control of the bed bugs is challenging but the nature of the condition should be carefully explained, and the patient and family empowered to eradicate the insect. Hygienic-environmental measures include a deep cleaning of the house, personal and bed clothes; and the eradication of the bed bugs through the application of insecticides (pyrethrin, permethrin, organophosphates and carbamates). Thirdly, medical treatment is symptomatic. If there is superadded infection, an antiseptic or topical or systemic antibiotic is used dependent on the extent and severity of the infection. For acute bites mild steroids, such as hydrocortisone, are recommended according to Mexican guidelines (17). Oral antihistamines are given for intense itching. The preventive use of repellents, such as citronella fragrances or 5% benzyl benzoate, help to reduce bites while the total eradication takes place (18).

Immunotherapy for papular urticarias caused by insect bites (mosquito) has been shown to be effective in improving skin lesions and increasing levels of the subclass of IgG4 that may have a protective role against subsequent reactions to exposures to the same insects (19).

Materials and methods

We recruited children aged 4-15 years old, from the allergy clinic at Hospital General de Mexico Eduardo Liceaga, an urban, tertiary referral center. We identified children who had recurrent papular urticaria caused by bed bugs in whom multiple previous treatments had been used without response (eradication of the bed bugs through the application of insecticides in their homes, topical corticoids, antihistamines). Written consent and written assent were obtained from the parents and child. Ethics approval was given by the Internal Committee of Hospital Bioethics.

The participants who fitted the selection criteria underwent an initial allergy assessment including clinical history, SPT and specific IgE to determine bed bug sensitization. Additional aeroallergens that were tested included mosquito, flea, *Dermatophagoides pteronyssinus* house dust mite, *Periplaneta americana*, *Alternaria* mould, *Aspergillus niger*, *Amaranthus palmeri*, *Atriplex bracteosa, Chenopodium album,* Salsola Kali*, Fraxinus americana, Ligustrum, Artemisia* spp., *Ambrosia* spp., *Cosmos bipinnatus, Hellianthus annus*, *Quercus* spp., *Alnus* spp., *Prosopis* spp., *Schinus molle*, *Populus alba, Cynodon dactylon*, *Lolium perenne*, *Phleum pratense,* cat and dog dander based on the Mexican immunotherapy guidelines (20). Prick testing was performed according to the method of the subcommittee on Skin Test of the American Academy of Allergy, Asthma & Immunology using standardized lancets. The participants were interrogated and classified in a socioeconomic stratum based on the Mexican Association of Research Agencies and Public Opinion A.C. (AMAI) in High Class (A/B), High Middle Class (C+), Middle Class (C), Middle Low Class (D+), Low Class (D), Extreme Poverty (E).

Twenty children were randomized to receive subcutaneous specific immunotherapy with a whole-body bed bug extract or conventional treatment (antihistamines, topical steroids, citronella fragrance).

C. lectularius extract was prepared from 4 g of dried *C. lectularius* were obtained from three homes of families affected by bed bugs in

Mexico City and from the Penitentiary Center ("Reclusorio Preventivo Varonil Norte") in Mexico City, by degreasing the samples with sulfuric ether and subsequently drying in the sun for a period of 12 hours and grinding them to powder using mortar and pestle. The bed bug allergen preparations were based on Good Manufacturing Practice with the references given in the work of Price *et al*. in Journal Allergy Clinical Immunology in 2012. The allergenic extract of bed bug was defined as 100 I.R./ml. (67 mcg/ml).
Immunotherapy was initiated with the whole-body bed bug extract at a concentration of 0.0001 wt/vol given subcutaneously. Thereafter biweekly subcutaneous injections, of progressively increasing doses were administered for three months (induction phase). The dose was then maintained after 12 weeks of treatment (maintenance phase) till the end of the study period (**table I**).

Table I *- Subcutaneous specific immunotherapy scheme.*

Induction dose	Schedule (Biweekly)	Week
0.001 wt/vol	0.1	1
	0.2	1
	0.4	2
	0.8	2
0.01 wt/vol	0.1	3
	0.2	3
	0.4	4
	0.8	4
0.1 wt/vol	0.1	5
	0.2	5
	0.4	6
	0.8	6
1 wt/vol	0.1	7
	0.2	7
	0.4	8
	0.8	8
10 wt/vol	0.1	9
	0.2	9
	0.4	10
	0.8	10
100 wt/vol	0.1	11
	0.2	11
	0.4	12
Maintenance Dose	**Weekly**	
50 mcg/ml	0.50 cc	

Mixed non-standardized whole-body extract of *C. lectularius* (bed bug).

The response to immunotherapy was assessed using scores of quality of life (Dermatology Quality of Life Index), scores of satisfaction to immunotherapy (ESPIA questionnaire) and intensity scores of pruritus (12-Item Pruritus Severity Scale). These were performed before the start of immunotherapy, at 3 months, at 6 months and at 12 months of treatment.
The DLQI score range is 0-30, the higher the score the higher the impairment of the QOL. Scores of 0-1 are defined as having no effect on the patient´s QOL, scores of 2-5 a small effect, scores of 6-10 a moderate effect, scores of 11-20 a very large and scores of 21-30 extremely large effects on patient´s QOL.
The intensity of pruritus was assessed using the 12-Item Pruritus Severity Scale. The score ranges from 3 (minimal pruritus) to 22 (most severe pruritus).
The satisfaction of treatment with immunotherapy was evaluated based on the ESPIA questionnaire that consists of 16 items distributed in 4 dimensions: perceived efficacy, activities and environment, cost-benefit balance, and general satisfaction. The final score ranges from 0 (low satisfaction) to 100 (high satisfaction).

Statistical analysis

Statistical evaluation was done by Mann-Whitney U-Test (two tailed probabilities), for the intergroup comparisons and the Wilcoxon signed rank for intragroup comparison at the different time of observation. The chi-square test was used to test the significance of differences among the overall evaluation stated at the end of the trial. The chosen level of significance was $p < 0.05$.

Results

A total of 20 patients were included for this study from 24 recruited, corresponding to a response rate of 83.3%. Three patients did not meet inclusion criteria (response to conventional treatment) and 1 patient refused participation. All 20 enrolled patients had recurrent papular urticaria caused by bed bugs which was not responsive to multiple previous treatments and which was affecting QOL. The mean age of the recruited children was 6.2 years (SD 1.73) and 100 % (n = 20) were male, none of the females having met inclusion criteria. A mean DLQI score of 23.60 (SD 2.34) and mean 12 item PSS score of 20.35 (SD 1.3) were recorded for the group on enrollment.
Twenty patients were randomized by coin toss, 12 to receive immunotherapy and 8 to receive conventional treatment. There were no significant differences between the two groups with regard the age, IgE (median 121 kU/l *versus* 140 kU/l), socioeconomic stratum, comorbidities and positive skin prick tests to aeroallergens (**table II**).

Quality of Life

Patients who received immunotherapy showed an improved mean DQLI score of 23.83 (SD 2.51) before starting treatment to 4.00 (SD 1.41) after 12 months of treatment. Compared to

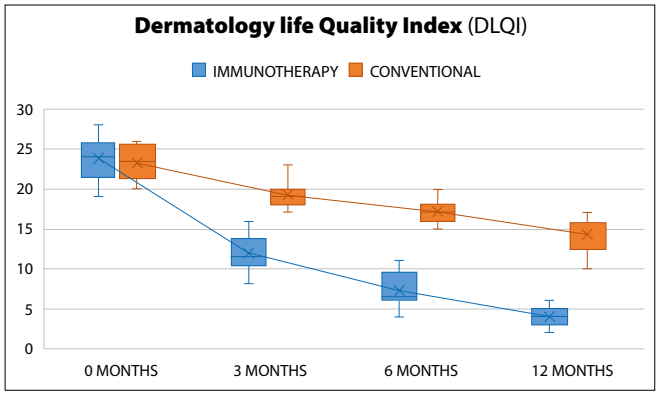
Table II - *Demographic characteristics of experimental group and control group.*

N	G	Age	Address	SEST	IgE	Other allergens SPT	Comorbidities	DLQI				12I PSS				ESPIA
								0	3	6	12	0	3	6	12	93
Experimental group																
1	M	8	**Mexico City, Iztapalapa**	D+	45	PER, DER	AR	21	14	8	5	20	10	8	4	94
2	M	6	**Mexico City, Iztapalapa**	D+	80	PER, MOS	None	25	13	11	6	22	12	6	5	96
3	M	4	**Mexico City, Gustavo A Madero**	D	67	DER, CYN, LOL	None	26	12	8	4	19	11	7	3	100
4	M	6	**Mexico City, Gustavo A Madero**	D	167	DER, MOS	None	24	10	6	4	20	8	6	3	100
5	M	7	**Mexico City, Gustavo A Madero**	D	79	None	None	19	8	4	2	21	14	10	4	88
6	M	8	**Mexico City, Iztacalco**	D+	212	AMA, FRAX, FEL	None	23	16	11	5	22	10	10	8	96
7	M	5	**Mexico City, Iztacalco**	D+	95	FLEA	AD	21	12	6	3	18	8	6	4	96
8	M	9	**Mexico City, Iztacalco**	D+	< 5	CAN, FEL	None	24	11	6	2	21	12	8	3	98
9	M	5	**Mexico, Naucalpan**	C	345	DER, PER, CYN	AR	25	11	7	5	20	14	10	3	96
10	M	10	**Mexico, Naucalpan**	C	98	None	None	28	9	4	3	20	9	5	4	93
11	M	7	**Mexico, Tlalnepantla**	D+	188	CAN, FEL	None	26	16	10	6	21	15	7	4	94
12	M	5	**Mexico, Tlalnepantla**	D+	75	None	None	24	11	6	3	22	14	8	3	94
Control group																
1	M	5	**Mexico City, Cuautemoc**	C	< 5	None	None	26	20	18	15	20	15	12	8	DNA
2	M	7	**Mexico City, Cuautemoc**	C	245	FRAX, HEL, CYN	AR	24	18	16	17	18	16	12	10	DNA
3	M	6	**Mexico City, Alvaro Obregon**	D	130	CAN	None	26	17	18	15	19	14	13	14	DNA
4	M	5	**Mexico City, Alvaro Obregon**	D	56	DER, PER	AD	23	19	16	12	21	13	10	6	DNA
5	M	8	**Mexico City, Alvaro Obregon**	D	78	None	None	20	18	15	10	22	18	15	15	DNA
6	M	4	**Mexico City, Alvaro Obregon**	D	53	None	None	22	20	16	14	20	16	12	13	DNA
7	M	5	**Mexico, Azcapotzalco**	D+	200	DER, PER	AR	24	23	20	15	22	16	12	10	DNA
8	M	4	**Mexico, Ecatepec**	D	353	FRAX, AMA, QUER, ALN, CYN	AR	21	19	18	16	19	14	10	8	DNA

N: number; G: gender; M: male; F: female; SEST: socioeconomic stratum; E: extreme poverty; D+: medium-low class; D: poverty; C: medium class; C+: medium-high class; A/B: high class; PER: *Periplaneta*; DER: *Dermatophagoides;* CYN: *Cynodon*; MOS: Mosquito; AMA: A*maranthus*; FRAX: *Fraxinus*; QUER: Q*uercus*; ALN: *Alnus*; HEL: *Hellianthus*; LOL: *Lollium*; CAN: dog; FEL: cat; AR: allergic rhinitis; AD: atopic dermatitis; SPT (Skin Prick Test); 12I-PSS: 12-Item Pruritus Severity Scale; DQLI: Dermatology Quality of Life Index; ESPIA: Satisfaction Immunotherapy Questionnaire; DNA: Did Not Apply.

patients receiving conventional treatment who showed an initial mean DLQI score of 23.25 (SD 2.18) and 14.25 (SD 2.25) after 12 months of treatment. Overall, the reduction in the DLQI score of 19.83 in the immunotherapy group *versus* 9 in the conventional treatment group was significant (p = 0.0012) (**figure 1**).

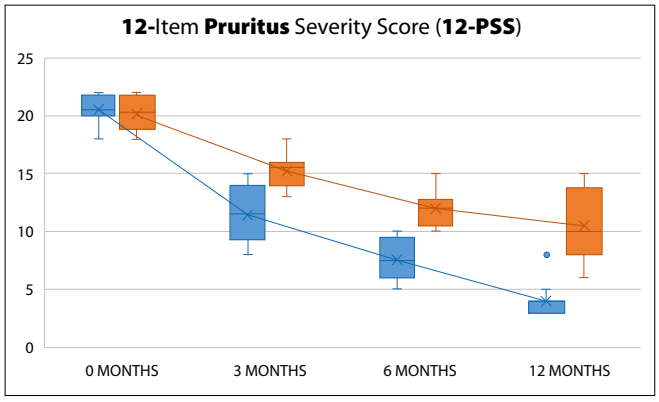
Figure 1 *- Dermatology Life Quality Index (DLQI).*



Intensity of pruritus

Patients who received immunotherapy experienced a reduction in the intensity of pruritus evaluated with the 12-Item Pruritus Severity Score (12-IPSS) from 20.5 (SD 1.24) to 4 (SD 1.41) after 12 months of treatment. Compared to patients with conventional treatment who presented an initial 12-IPSS of 20.13 (SD 1.45) and a final of 10.5 (SD 3.4) after 12 months. Overall, the reduction in the 12-IPSS of 16.5 in the immunotherapy group *versus* 9.63 in the conventional treatment group was significant (p = 0.02) (**figure 2**).

Figure 2 *- 12-Item Pruritus Severity Score (12-PSS).*



The satisfaction of the immunotherapy was assessed using the ESPIA Questionnaire and a mean satisfaction of 95.75 (SD 3.3) was recorded.

Figure 3 *- Evolution of skin lesions.*

The yearly cost analysis of the immunotherapy was $ 60.00 on average for each patient which was accompanied by a reduction in the conventional medicines (antihistaminics, steroids, insect repellents) used, compared to the control group receiving conventional treatment with an average cost of $ 180.00 for each patient without a decrease in the use of medications during the study period.

After twelve months of treatment, all 12 patients who received immunotherapy, reported a decrease of persistent cutaneous lesions (papular urticaria) (**figure 3**). On the contrary the other 8 patients with conventional treatment did not present a significant reduction of cutaneous lesions (papular urticaria).

Discussion

Papular urticaria is a manifestation of recurrent pruritic papules or vesicles and varying degrees of local edema. Reactions are thought to be the result of a hypersensitivity reaction to biting, stinging, or urticating insects (mosquitoes, flies, gnats, mites, ticks and bed bugs) (18).

Cuellar *et al.* demonstrated that papular urticaria was a chronic allergic disease where there was a genetic predisposition with an increased expression of molecules such as CD83, CD86 and HLA-DR which are related to antigen presentation and there are lower levels of regulatory cytokines such as interleukin-6 and IL-10 leading to an increase in the production of Th-2 cytokines ending in the production of a skin allergic reaction to exposure to an allergen in the sting or bite of an insect (saliva) (19).

Penneys *et al.* demonstrated human antibody binding to salivary gland and foregut endothelial protein antigen in mosquitoes. Previously sensitized sites also erupt following the appearance of new lesions, suggesting that circulating antigen triggers the reactivation of sensitized sites (20).

Price *et al.* demonstrate the development of an IgE response to *C. lectularius* following bed bug bites and Leverkus *et al.* identified the allergen as nitroforin in the bed bug's saliva (21).

Allergen specific immunotherapy (SIT) has been studied and used since Noon's first report in 1911. SIT is the only treat-

ment option that modifies fundamental allergic mechanism by inducing desensitization. Immunological changes associated with immunotherapy result in clinical tolerance (decrease in antigen-specific responsiveness) and immunologic tolerance (specific immune deviation from a TH2 to a TH1 cytokine profile). Until now, 6 studies on mosquito immunotherapy have been conducted based on clinical variables such as skin reactivity, nasal reactivity, symptom and drug scores and immunological variables such as increase in IgG4 antibody levels. No study has been carried out in bed bug immunotherapy. Both insects causes have been shown to be allergic hypersensitivity reactions to the saliva allergen found in both insects (22, 23).

In the present study patients in the active group receiving immunotherapy with whole-body bed bug (*C. lectularius*) extract for 1 year demonstrated significant improvement in clinical variables (skin lesions, improvement in quality of life, intensity pf pruritus and satisfaction with treatment) compared with the conventional treatment group.

In this study, with the progression of immunotherapy 100% of patients showed improvement in quality of life with a DQLI with a reduction from 23.86 to 11.91 (51%) in the Score at month 3 of treatment and from 23.86 to 4.13 (83%) at month 12 of treatment, being the maximum improvement in the first three months of treatment. The decrease in the intensity of pruritus was 44% in the third month of treatment and 80% after 12 months of treatment being the maximum intensity of pruritus reduction in the first three months of treatment.

Satisfaction with immunotherapy using the ESPIA questionnaire was greater than 88 in the 12 patients with an average satisfaction of 95, supporting a high satisfaction with the treatment.

The medical treatment for bed bug papular urticaria is with topical steroids at the site of the bite and on acute lesions. The medical treatment for pruritus is with antihistamines. The most specific and curative management is the eradication of the bed bugs and thus exposure, but this can be challenging. Bed bugs are very resistant insects, that can survive for up to a year without food and are able to extend their territory through walls and ceilings. Eradication is best performed by a professional, but this is expensive often a change in household furniture is recommended and thus an unaffordable option for the vast majority of affected patients (21).

Limitations

The limitations of this study include small sample size recruited in a tertiary allergy clinic, which makes it difficult to extrapolate results to the general population. *In vitro* tests are not available to identify specific IgE against bed bugs saliva antigens. The treatment is focused on the reduction of symptoms and improvement in quality of life, does not reduce the transmission of the disease therefore it does not replace the definitive treatment that is the extermination of the bed bugs.

Conclusions

This pilot study demonstrates that subcutaneous immunotherapy with a whole body, bed bug extract is effective in reducing the number of skin lesions and pruritus intensity while improving quality of life in patients with recurrent papular urticaria caused by bed bug bites who had failed multiple previous adequate and appropriate treatments. It was rated as a highly satisfactory treatment by patients.

The objective of this preliminary study was to determine the feasibility for a larger, future study in collaboration with first level services focused on populations of low socioeconomic level, with a large number of patients using a standardized extract.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Thomas I, Kihiczak GG, Schwartz RA. Bedbug bites: a review. Int J Dermatol. 2004;43(6):430-3. doi: 10.1111/j.1365-4632.2004.02115.x.
2. Lozano AM, López JF, Zakzuk J, García E. Papular urticaria: A review of causal agents in Colombia. Biomedica. 2016;36(4):632-45. doi: 10.7705/biomedica.v36i4.3258.
3. Panagiotakopulu E, Buckland P. Cimex lectularius L., the common bed bug from Pharaonic Egypt. Antiquity. 1999;73:908-911. Available at: https://www.academia.edu/6015337/Panagiotakopulu_E_Buckland_P_C_1999_Cimex_lectularius_L_the_common_bed_bug_from_Pharaonic_Egypt_Antiquity_73_908_911.
4. Del Pozzo-Magaña BR, Lazo-Langner A, Gutiérrez-Castrellón P, Ruiz-Maldonado R. Common Dermatoses in Children Referred to a Specialized Pediatric Dermatology Service in Mexico: A Comparative Study between Two Decades. ISRN Dermatol. 2012;2012:351603. doi: 10.5402/2012/351603.
5. Rahlenbeck S, Utikal J, Doggett S. On the Rise Worldwide: Bed Bugs and Cimicosis. BJMP. 2016;9(3):a921. Available at: https://www.bjmp.org/content/rise-worlwide-bed-bugs-and-cimicosis.
6. Recommendations for the management of bed bugs in New York City: New York City Bed Bug Advisory Board Report to the Mayor and City Council. New York City, 2010.
7. Wang C, Wen X. Bed Bug Infestations and Control Practices in China: Implications for Fighting the Global Bed Bug Resurgence. Insects. 2011;2(2):83-95. doi: 10.3390/insects2020083.
8. Doggett S, Geary M, Russell R. Doggett S, Geary M, Russell R. The resurgence of bed bugs in Australia: with notes on their ecology and control. Environ Health 2004;4(2):30-8. Available at: https://www.semanticscholar.org/paper/The-Resurgence-of-Bed-Bugs-in-Australia%3A-With-Notes-Doggett-Geary/59e1d7c446e904194bb5448c271d5b38c032db77.
9. Romero A. Moving From the Old to the New: Insecticide Research on Bed Bugs since the Resurgence. Insects. 2011;2(2):210-7. doi: 10.3390/insects2020210.
10. Reinhardt K, Kempke D, Naylor RA, Siva-Jothy MT. Sensitivity to bites by the bedbug, Cimex lectularius. Med Vet Entomol. 2009;23(2):163-6. doi: 10.1111/j.1365-2915.2008.00793.x.

11. Goddard J, deShazo R. Bed bugs (Cimex lectularius) and clinical consequences of their bites. JAMA. 2009;301(13):1358-66. doi: 10.1001/jama.2009.405.
12. Criado PR, Criado RF. Bedbugs (Heteroptera, Cimicidae): an etiology of pruritus to be remembered. An Bras Dermatol. 2011;86(1):163-4. English, Portuguese. doi: 10.1590/s0365-05962011000100028.
13. Lavery MJ, Stull C, Kinney MO, Yosipovitch G. Nocturnal Pruritus: The Battle for a Peaceful Night's Sleep. Int J Mol Sci. 2016;17(3):425. doi: 10.3390/ijms17030425.
14. Golden DB, Moffitt J, Nicklas RA, Freeman T, Graft DF, Reisman RE, *et al*. Stinging insect hypersensitivity: a practice parameter update 2011. J Allergy Clin Immunol. 2011;127(4):852-4.e1-23. doi: 10.1016/j.jaci.2011.01.025.
15. Price JB, Divjan A, Montfort WR, Stansfield KH, Freyer GA, Perzanowski MS. IgE against bed bug (Cimex lectularius) allergens is common among adults bitten by bed bugs. J Allergy Clin Immunol. 2012;129(3):863-5.e2. doi: 10.1016/j.jaci.2012.01.034.
16. Leverkus M, Jochim RC, Schäd S, Bröcker EB, Andersen JF, Valenzuela JG, *et al*. Bullous allergic hypersensitivity to bed bug bites mediated by IgE against salivary nitrophorin. J Invest Dermatol. 2006;126(1):91-6. doi: 10.1038/sj.jid.5700012.
17. Larenas-Linnemann D, Medina-Ávalos MA, Ortega-Martell JA, Beirana-Palencia AM, Rojo-Gutiérrez MI, Morales-Sánchez MA, *et al*. Guía Mexicana para el Diagnóstico y el Tratamiento de la Urticaria [Mexican guidelines on the diagnosis and treatment of urticaria]. Rev Alerg Mex. 2014;61 Suppl 2:S118-93. Spanish. Available at: https://pubmed.ncbi.nlm.nih.gov/25724222/.
18. Boase C. Bedbugs back from the brink. Pestic Outlook. 2001;12:159-62. doi: 10.1039/B106301B.
19. Srivastava D, Singh BP, Sudha VT, Arora N, Gaur SN. Immunotherapy with mosquito (Culex quinquefasciatus) extract: a double-blind, placebo-controlled study. Ann Allergy Asthma Immunol. 2007;99(3):273-80. doi: 10.1016/S1081-1206(10)60664-3.
20. Larenas-Linnemann D, Luna-Pech JA, Rodríguez-Pérez N, Rodríguez-González M, Arias-Cruz A, Blandón-Vijil MV, *et al*. GUIMIT 2019, Guía Mexicana de Inmunoterapia. Guía de diagnóstico de alergia mediada por IgE e inmunoterapia aplicando el método ADAPTE [GUIMIT 2019, Mexican Guideline on Immunotherapy. Guideline on the diagnosis of IgE-mediated allergic disease and immunotherapy following the ADAPTE approach]. Rev Alerg Mex. 2019;66 Suppl 1:1-105. Spanish. doi: 10.29262/ram.v66i5.631.
21. Heng MC, Kloss SG, Haberfelde GC. Pathogenesis of papular urticaria. J Am Acad Dermatol. 1984;10(6):1030-4. doi: 10.1016/s0190-9622(84)80330-8.
22. Cuéllar A, García E, Rodríguez A, Halpert E, Gómez A. Functional dysregulation of dendritic cells in patients with papular urticaria caused by fleabite. Arch Dermatol. 2007;143(11):1415-9. doi: 10.1001/archderm.143.11.1415.
23. Penneys NS, Nayar JK, Bernstein H, Knight JW. Circulating antibody detection in human serum to mosquito salivary gland proteins by the avidin-biotin-peroxidase technique. J Am Acad Dermatol. 1988;18(1 Pt 1):87-92. doi: 10.1016/s0190-9622(88)70013-4.
24. Divjan A, Price JB, Acosta LM, Rundle AG, Goldstein IF, Jacobson JS, *et al*. Development Of IgE Against a Cimex Lectularius Allergen After Being Bitten By Bed Bugs Was Common Among Children In NYC. J Allergy Clin Immunol 2014;133. doi: 10.1016/j.jaci.2013.12.593.
25. Ariano R, Panzani RC. Efficacy and safety of specific immunotherapy to mosquito bites. Eur Ann Allergy Clin Immunol. 2004;36(4):131-8. Available at: https://pubmed.ncbi.nlm.nih.gov/15180354/.
26. Manrique MA, González-Díaz S, Arias-Cruz A, Hernandez M, Gallego C, Garcia-Calderin D, *et al*. 463 Efficacy of Immunotherapy With Allergenic Extract of Aedes Aegypti in the Treatment of Large Local Reaction to Mosquito Bites in Children. World Allergy Organization J. 2012;5S164. doi: 10.1097/01.WOX.0000411578.60734.e0.

Eur Ann Allergy Clin Immunol Vol 54, N.6, 265-276, 2022

I. Alen Coutinho[1*], F. Costa Sousa[2,3*], F. Cunha[1], C. Frutuoso[2], C. Ribeiro[1], C. Loureiro[1], F. Águas[2], A. Todo Bom[1,4]

Key elements in hypersensitivity reactions to chemotherapy: experience with rapid drug desensitization in gynaecological cancer in a Tertiary Hospital

[1]Department of Allergy and Clinical Immunology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[2]Department of Gynecology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[3]Coimbra Clinical Academic Center, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[4]Institute of Pathophysiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
*These authors contributed equally to the work, being the first two authors of the manuscript

Key words

Adults; chemotherapy hypersensitivity reactions; gynaecological cancer; rapid desensitization; risk factors.

Corresponding author
Iolanda Alen Coutinho
Immunoallergology Service
Centro Hospitalar Universitário de Coimbra
Praceta Prof. Mota Pinto
3000-075 Coimbra, Portugal
ORCID ID: 0000-0002-2511-4843
E-mail: iolandaalen@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.207

Summary

Rapid drug desensitization (RDD) is a procedure performed when no alternative drug is considered equally effective. The aim of our study is to describe the experience with RDD to cytostatics in patients being treated for gynaecological cancer in a Tertiary Hospital, over a period of 5 years. In this paper, we review 22 cases and 107 episodes of RDD; 86.3% of patients had advanced disease and the mortality rate at the time of data collection was 50.0%. RDD was performed on 81.8% patients for platinum, 13.6% for taxanes, and 4.5% for anthracyclines. The reintroduction of antineoplastic drugs in all patients with a previous history of immediate hypersensitivity reaction demonstrated the safety and efficacy of this procedure. There was serious complication (anaphylaxis) in only one case.

Impact statement

This work highlights the safety and effectiveness of antineoplastics.

Introduction

Considering developed countries, the most lethal gynaecological cancer is ovarian, especially high-grade serous carcinoma (1). Ovarian cancer is usually diagnosed at advanced stages (stage III and IV of The International Federation of Gynecology and Obstetrics – FIGO) (1, 2), with 5-year survival rates in all stages reported as low as 50% (3). Platinum-based therapeutic agents are widely used in this subtype, due to a generally good response (1). Other histological types like low-grade serous or clear cell ovarian carcinomas do not show such a good clinical response to these therapies (1) but can still be used in these cases. Platinum sensitivity is described as an absence of relapse for at least 6 months after the last cycle of chemotherapy (4). Nevertheless, platinum agents also play an important role in subsequent relapses of ovarian cancer, as sensitivity is often retained (4). Paclitaxel is often used together with platinum drugs, as this combination improves overall survival in patients with recurrent platinum-sensitive ovarian cancer (5). Platinum-related hypersensitivity reactions (HSR) are reported at a rate of 1 in 10 patients, with higher incidence in advanced stages of the disease (stages III-IV), with serous carcinoma subtype and in the pres-

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

ence of ascites (5). Multiple cycles of platinum therapies increase the probability of an HSR (4, 5), so desensitization protocols can enhance the chance of retreatment with these drugs and thus increase patient survival.

In developed countries, endometrial cancer is the most common gynaecological malignancy (6). Most of these tumours have a postmenopausal diagnosis and are usually detected at stage I (with > 95% survival rates at 5 years), although they can appear before 40 years of age (6, 7). Nevertheless, if there is locoregional advanced or distant disease, survival rates decrease markedly (8). There are no population-based screening programmes for endometrial or ovarian cancer. Among those with high-risk factors for endometrial tumours, only those with Lynch syndrome have customized screening programmes.

Three of the main histological endometrial cancer subtypes according to the World Health Organization (WHO) include endometrioid, serous, and clear-cell carcinomas (9). Classically and biologically, endometrial carcinomas are divided into 2 types, with type 2 referring to those with typically worse prognosis and including high-grade carcinomas (7, 9).

FIGO classification is used for staging endometrial tumours; the staging is surgical (10). Additionally, a risk group stratification classifies the following cases as a high-risk disease: endometrioid IB G3; stage ≥ II; and non-endometrioid histological subtype (11). Chemotherapy is recommended in endometrioid stage III of FIGO and the approved regime is carboplatin plus paclitaxel. The same therapy is proposed for serous and clear-cell carcinomas (8, 12).

Worldwide, breast cancer is the main cause of cancer-related deaths in women (13). The incidence of breast cancer increases with age (only 25% of cases occur before age 50) and has increased due to the application of national screening programmes with the use of mammography (13). As increasing numbers of breast cancer cases are diagnosed at an earlier stage, the mortality rate has decreased in developed countries in recent years (13). The histological diagnosis is based on WHO (World Health Organization) classification and the eighth edition of the American Joint Committee on Cancer (AJCC) tumour, node, metastases (TNM) staging system. These include biological prognostic information: grade, hormonal receptors, human epidermal growth factor receptor 2 (HER 2) and possibly gene expression (13). Of all the breast cancer subtypes, invasive carcinomas not otherwise specified (NOS) are the most frequent (13).

Hypersensitivity reactions to chemotherapeutic agents

HSRs to a chemotherapy agent are defined as unexpected reactions that cannot be explained by the known toxicity profile of the drug (14). Based on their development mechanism, HSRs are classified as allergic, which involves an immunological mechanism, and non-allergic, when an immunological pathogenic mechanism is not demonstrated (15). The type of allergic reaction is classified into four categories based on the Gell and Coombs classification: type I immunoglobulin E (IgE)-mediated or immediate type; type II cytotoxic-mediated; type III immune complex-mediated; and type IV T cell-mediated or delayed type (16).

Current data indicate that most patients who have experienced HSRs within 24 hours of the last drug administration achieve drug-tolerance with rapid desensitization (15). Immediate drug HSRs usually occur within 1–6 h after the last administration of the drug, and can be IgE-mediated or related to a non-specific histamine release. Non-immediate or delayed reactions can occur at any time from 1 h after the initial drug administration, typically up to 6-12 h. Specifically in cases of chemotherapy, HSRs can occur more than 24 h after drug infusion, probably due to its prolonged half-life, or the administration of premedication drugs, which can mask the acute phase of these reactions (18, 19).

Platinum compounds

Platinum compounds, such as cisplatin, carboplatin and oxaliplatin, are useful antineoplastic agents used in a wide variety of cancers, particularly in gynaecological malignancies (20-22). The first platinum drug approved by the United States Food and Drug Administration (FDA) as an anticancer agent was cisplatin in 1970, with carboplatin being approved almost twenty years later in 1989 (16). These chemotherapy agents are classified as deoxyribonucleic acid (DNA) alkylating agents. However, cisplatin was gradually replaced by carboplatin in patients with ovarian cancer, due to its reduced nephrotoxicity, neurotoxicity and gastrointestinal toxicity (23, 24). Oxaliplatin is a third-generation platinum agent that, similarly to cisplatin and carboplatin, consists of a DNA alkylating agent that forms intrastrand/interstrand DNA crosslinks, affecting DNA base pairing, replication, gene transcription, and cell death (25, 26). The mechanism by which platinum compounds cause HSRs remains unclear, but they are generally reported as being IgE-mediated reactions (16, 24, 27). In the available studies regarding the incidence of HSRs to platinum compounds: cisplatin HSRs range from 1% to 20%; carboplatin HSRs increase with the number of cycles, and range from 1% in those that received ≤ 6 cycles to 27% in those who received ≥ 7 cycles, and up to 47% in those who received ≥ 15 cycles; and oxaliplatin HSRs range from 10% to 25% (26, 28-30). The incidence of cross-reactivity between platinum agents has yet to be clarified, but some studies have documented the cross-reactivity between carboplatin and cisplatin as being higher than 25% (31). The clinical manifestations of HSRs are diverse and unpredictable, varying from only cutaneous manifestations to severe or even fatal manifestations (26, 32).

Taxanes

Similar to platinum compounds, taxanes are an important cause of HSRs in oncologic patients. The most used taxanes are paclitaxel and docetaxel. Paclitaxel is a natural compound, originally

isolated from the bark of the Pacific yew tree, with its antineoplastic effect interfering with the dynamics of microtubules (cytoskeleton), causing mitotic block and cell death (33-35). Docetaxel is a semi-synthetic molecule derived by a taxoid precursor found in European yew trees (35). Due to their low solubility, they are formulated with solvents to allow intravenous administration: Cremophor® EL is associated with paclitaxel and polysorbate 80 with docetaxel (33). These solvents are capable of causing complement activation which leads to anaphylatoxins production and mast cell activation, thereby explaining why some patients experience immediate HSRs (34). Initial studies with taxanes revealed a high incidence of immediate HSRs, which led to the use of premedication with antihistamine and corticosteroids (36). Currently, immediate HSRs to paclitaxel and docetaxel occur in about 10% and 5% of premedicated patients, respectively (36). Immediate HSRs to taxanes occur minutes after starting the infusion during the first or second cycles, and symptoms include flushing, chest, back and abdominal pain, as well as respiratory symptoms (33, 35). Cross-reactivity between paclitaxel and docetaxel exists but seems to vary among different populations and depends on the severity of the initial HSR (32).

Anthracyclines

Anthracyclines, such as doxorubicin, idarubicin, daunorubicin and epirubicin, are used to treat multiple malignancies, interfering with DNA metabolism and ribonucleic acid (RNA) production. HSRs to anthracyclines are rare (32). Clinically, the most important anthracycline that has been well studied, particularly in gynaecological malignancies, is pegylated liposomal doxorubicin (PLD) (32). The reported incidence of immediate HSRs to PLD is 9% (16). This is similar to the incidence of HSRs to taxanes and usually occurs at an interval of 5 minutes after the first cycle, with flushing, back pain and chest tightness as clinical manifestations (16). The mechanism of HSRs is not clear, but it is believed that symptoms derive from complement and mast cell activation (37). Also, interestingly, the free form of doxorubicin does not cause HSRs, with pegylated liposomes being the probable trigger for these reactions (37).

Risk factors to hypersensitivity reactions in chemotherapeutic agents

The main risk factor influencing the occurrence of platinum HSRs is the total number of cycles that patients have received, with the peak of HSRs usually occurring during the 8th or 9th cycle (32). Similarly, other risk factors have been reported, namely a history of atopy and drug allergy; a long platinum-free interval and the administration of ≥ 650 mg of carboplatin (32). The inherited mutations in breast cancer type 1 or 2 genes (Breast Cancer gene – BRCA 1 or 2) appear to be related to a higher risk of reactions to carboplatin infusion and patients are also at risk for these reactions during desensitization (23). On the other hand, the combination of carboplatin with PLD seems to reduce the incidence of HSRs when compared to the administration of isolated carboplatin or its combination with paclitaxel (26). There are studies suggesting that the combination of specific chemotherapeutic drugs may have a predictive value on the HSRs risk (26, 38). Joly *et al.* suggested that the use of carboplatin associated with pegylated liposomal doxorubicin presented a low rate of HSRs, when compared to the combination with paclitaxel, which seems to be associated with a higher number of HSRs, probably due to potentiation secondary to paclitaxel co-administration (26, 38). The predictive factors for HSRs in the case of taxanes remain unclear. However, one study identified that younger age, previous allergy history and a short-course of premedication were associated with paclitaxel HSRs (39). Other comorbidities, such as obesity (body mass index > 30 kg/m^2) have also been associated with an increased risk of HSR to chemotherapy agents (40).

The influence of the presence of eosinophils in allergic diseases is already known, having been studied as a possible risk factor for drug reactions, especially regarding their count in the platinum therapeutic cycle in which the allergic reaction occurred (41). However, some studies carried out in this area have proven an absence of relationship or the presence of a lower number of eosinophils in patients with reactions to platinum salts when compared with non-allergic patients (41).

Rapid drug desensitization

Rapid drug desensitization (RDD) is a procedure performed when no alternative drug in use is deemed equally effective. RDD consists in the induction of temporary unresponsiveness to drug antigens, which allows patients to be treated with medications to which they have previously presented HSRs (42). RDD enables the full therapeutic doses to be reached without major side effects in a relatively short period of time (43). Several desensitization protocols have been published and used for patients with platinum drug HSRs, but most widely accepted desensitization protocols are the 8-step and 12-step, with a duration of 5.8 hours to 8 hours (22, 44, 45). The choice of a specific RDD protocol is based on the risk stratification, according to clinical history and skin test results (46). Various desensitization protocols for taxanes have been studied with the Brigham and Women's Hospital/Dana-Farber Cancer Institute, the 3-bag, 12-step protocol being the most studied as well as having an excellent safety record (32, 47). Regarding the PLD desensitization protocol, data are limited, but the most used protocol is the same as that used for taxanes, consisting of 3 bags and 12 steps (47).

The aim of chemotherapy desensitization is to maintain a temporary tolerance to the chemotherapeutic drug involved in the patient's reaction, which is essential for effective treatment and to achieve the best possible quality of life.

The objective of our study was to describe the experience in rapid drug desensitization to antineoplastic drugs in gynaecological cancers in an Allergy and Clinical Immunology Department of a Tertiary Hospital.

Materials and methods

Study design

The authors performed a retrospective, descriptive and inferential review of patients with gynaecological cancer with a history of HSRs to chemotherapy agents who were submitted to desensitization protocols.

Enrolment took place in a Tertiary Hospital over a period of five years, between June 2015 and June 2020. Patients were included if they had a histologically confirmed diagnosis of gynaecological cancer – ovarian, endometrial or breast, with subtype classification according to WHO guidelines (9, 48) – associated with confirmed HSRs to chemotherapy drugs (defined by European Academy of Allergy and Clinical Immunology – EAACI - guidelines (49)), and who were submitted to RDD. Patients were also required to be ≥ 18 years of age and able to provide written informed consent before each desensitization.

This paper was written considering the ethical and legal principles and in accordance with the recommendations of the Declaration of Helsinki of the World Medical Association. The anonymity of all the participants of this work was guaranteed.

Subjects

Patients included were those referred to the Allergy and Clinical Immunology Department by the Gynaecology Department, with immediate-type HSRs to chemotherapy drugs and an absence of possible alternatives, and who were eligible for allergy diagnostic work-up and RDD. Patients with delayed reactions (> 24 hours) were excluded, such as drug-induced fever and exfoliative skin reactions as multiform erythema, Stevens-Johnson syndrome or toxic epidermal necrolysis. Type II and III reactions (Gell and Coombs) were also excluded.

Immediate HSRs were classified according to Brown's grading system (BGS) (50) as: 1) Mild (grade I), corresponding to symptoms limited to the skin or involving a single organ/system; 2) Moderate (grade II), corresponding to symptoms involving at least two organs/systems without a significant drop in blood pressure or in oxygen saturation; 3) Severe (grade III), corresponding to symptoms involving at least two organs/systems and a significant drop in blood pressure or in oxygen saturation. Organs/systems signs and symptoms of HSRs were defined as mucocutaneous (flushing, pruritus, urticaria, angioedema), respiratory (nasal symptoms, dyspnoea, wheezing, oxygen desaturation, bronchospasm, throat or chest tightness), cardiovascular (chest pain, tachycardia, lipothymia, syncope, and hypotension), gastrointestinal (nausea, vomiting, diarrhoea, and abdominal pain) and other symptoms (altered state of consciousness, headache, paraesthesia, pain).

Disease characteristics and outcomes

Patients were characterized according to demographic data, history of atopy, age at diagnosis of gynaecological cancer, histological subtype of cancer, staging, distant metastases at diagnosis, drug and therapeutic cycle involved in HSR, time interval between cycles, Brown's grading system (BGS) (50) of HSR, drug chosen for RDD, number of therapeutic cycles in RDD, complications of RDD, the therapeutic success of RDD and mortality.

Assessment of allergy diagnosis

Each patient was evaluated with a detailed clinical history. Personal and family history of atopy were also considered.

All patients underwent skin tests for the suspected chemotherapeutic agent and latex at least 4 weeks after the initial reaction, according to The European Network for Drug Allergy/EAACI recommendations (49), with the exception of a single case of anthracycline reaction, where RDD was based only on clinical history due to the absence of standardization skin test concentrations. Skin prick tests (SPT) were performed with undiluted agents – carboplatin (10 mg/mL), cisplatin (1 mg/mL) (49), paclitaxel (6 mg/mL) and docetaxel (1 mg/mL) (51). When SPT were negative, intradermal tests (IDs) were performed with a 1/10 dilution and additional dilution in cases with moderate and severe reaction (grade II and III (50)). Immediate readings were performed at 15-20 minutes for SPT and IDs (49). A weal of ≥ 3 mm in diameter for SPT or an increase in diameter of the initial ID of ≥ 3 mm were defined as a positive result, when observed a negative response to control solution (0.9% saline) and a positive response to histamine (10 mg/mL) (49).

Desensitization protocol

A 12-step protocol, described by Castells *et al.* (42) was implemented, with three dilutions of the target drug dose, containing 1/100, 1/10, and 1/1, respectively, diluted in 250 mL of 0.9% saline solution.

All desensitization procedures were conducted on an inpatient regimen and were performed using premedication: clemastine 2 mg, intravenously; ranitidine 50 mg, intravenously, and montelukast 10 mg, per os, maintaining the premedication proposed by oncology according to the drug or the protocol used. Based on our experience and adapted from the literature, our RDD protocols include premedication administration 30 minutes before starting the procedure, with the exception of montelukast, which was administered in 2 steps: 12 h and 30 minutes before the procedure (52).

Angiotensin-converting enzyme inhibitors and beta-adrenergic blockers were retained for 24 hours before desensitization.

The treatment of adverse drug reactions depended on the severity: mild reactions were treated with cetirizine (10 mg administered per os), and moderate-severe/recurrent reactions were treated with clemastine (2 mg intravenously or intramuscularly), ranitidine (50 mg administered intravenously), and methylprednisolone sodium succinate (1-2 mg/kg administered intravenously). Bronchospasm was also treated with inhaled beta2 agonists (salbutamol or ipratropium bromide). In anaphylactic reactions, epinephrine (0.01 mg/kg intramuscular at the dilution of 1 mg/mL) was administered, corresponding, in our sample, to 1 case.

Upon resolution, infusion was continued at the current step, or at a previous step, depending on the severity of the reaction (53).

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 24.0®. Descriptive statistics were analysed as mean and standard deviation for the variables with normal distribution, and median and interquartile range for variables without normal distribution.

Results

Demographic and clinical characteristics

Twenty-two women with HSRs to chemotherapy agents were submitted to RDD, corresponding to 107 RDD procedures (mean 4.9 ± 4.5 procedures per patient). The mean age of the study cohort at the time of the first desensitization was 56.5 ± 14.3 years, ranging from 22 to 77 years old. Obesity (defined as body mass index > 30 kg/m^2) was observed in 31.8% (n = 7) and history of atopy was confirmed in 27.2 % (n = 6) patients: confirmed drug allergy history (nonsteroidal anti-inflammatory drugs) in n = 3 patients; allergic asthma and rhinitis in n = 2 patients; food allergy in n = 1. One third (n = 2) of the patients with a positive history of atopic disease had also a history of family atopy (allergic asthma or penicillin allergy).

Patient characteristics are summarized in **table I**.

Cancer diagnosis

Regarding diagnosis, the mean age of cancer diagnosis was 51.5 ± 12.8 years, ranging from 22 to 69 years old. Most of the patients (77.3%, n = 17) had advanced disease (stage III or IV) at the time of diagnosis. The types and subtypes of primary cancer are summarized in **table II**.

When retrospective data were collected, half of the patients, corresponding to n = 11, had already died due to disease progression. Of this group of patients, only one had a disease-free survival interval of more than one year. Considering patients diagnosed until 2015 (40.9%, n = 9), the 5-year survival rate was 55.6% (n = 5 of total n = 9).

Only 1 patient presented a BRCA 1 pathogenic mutation: a high-grade serous ovarian cancer and cisplatin HSR (patient number 16). Another patient presented a BRCA 2 variant of undetermined significance (VUS), this one with a low-grade serous ovarian cancer and carboplatin HSR (patient number 3); these two patients presented different severity of HSRs (grade III in patient number 16 and grade I in patient number 3).

Hypersensitivity reactions and desensitization

These data are summarized in **table I** and time intervals between lines of treatment are shown in **figure 1**, by patient and drug. Clinical manifestations of HSRs are illustrated in **figure 2**.

Platinum compounds: most patients (81.8%, n = 18) presented HSRs to platinum salts: 88.9% (n = 16 of total n = 18) to carboplatin and 11.1% (n = 2 of total n = 18) to cisplatin. Regarding comorbidities, one third of the patients (n = 6 of the total n = 18) were obese and 22.2% (n = 4 of the total n = 18) had atopy. According to BGS (50), HSRs were characterized as grade I in 27.8% (n = 5 of total n = 18), grade II in 5.6% (n = 1 of total n = 18) and grade III in 66.7% (n = 12 of total n = 18). The first episode of HSR to platinum occurred at a median 13.0 cycles (minimum 5 cycles, maximum 19 cycles). Almost all patients (94.4%, n = 17 of total n = 18) experienced the HSR in a number of cycles ≥ 7. The median platinum dose in the HSR cycle was 527.2 mg ± 161.2 mg, corresponding in 22.2% (n = 4 of total n = 18) to a dose ≥ 650 mg. Median blood eosinophil count before the administration of the HSR cycle was 98.3 cells/µl (minimum 0 cells/µl, maximum 300 cells/µl).

In patients diagnosed with ovarian cancer, the median time interval between the HSR and previous chemotherapy cycle was 10.7 months (minimum 2 months, maximum 23 months), and in 1 patient with ovarian cancer (patient number 15) platinum HSR occurred during the first group of cycles (5th cycle).

In patients diagnosed with endometrial cancer, the median time interval between the HSR and previous chemotherapy cycle was 49.0 months (minimum 8 months, maximum 144 months). In 1 patient with endometrial cancer (patient number 5) platinum HSR occurred during the first group of cycles cycle (8th cycle).

In 50.0% (n = 9 of total n = 18) of platinum chemotherapy HSRs, patients were under treatment with placlitaxel (skin tests confirmed platinum hypersensitivity).

Skin prick tests (SPT) with platinum compounds were positive in 38.9% of the patients (n = 7 of total n = 18), all to carboplatin (n = 4 grade III and n = 3 grade I). In all patients with positive SPT, IDs were not performed due to a significant risk of HSRs associated to the procedure. ID tests were positive in 55.5% (n = 10 of total n = 18), n = 7 carboplatin and n = 2 cisplatin (n = 1 grade I; n = 2 grade II; n = 6 grade III). Evidence of skin test cross reactivity had been presented in 4 patients (n = 2 cisplatin, n = 2 carboplatin).

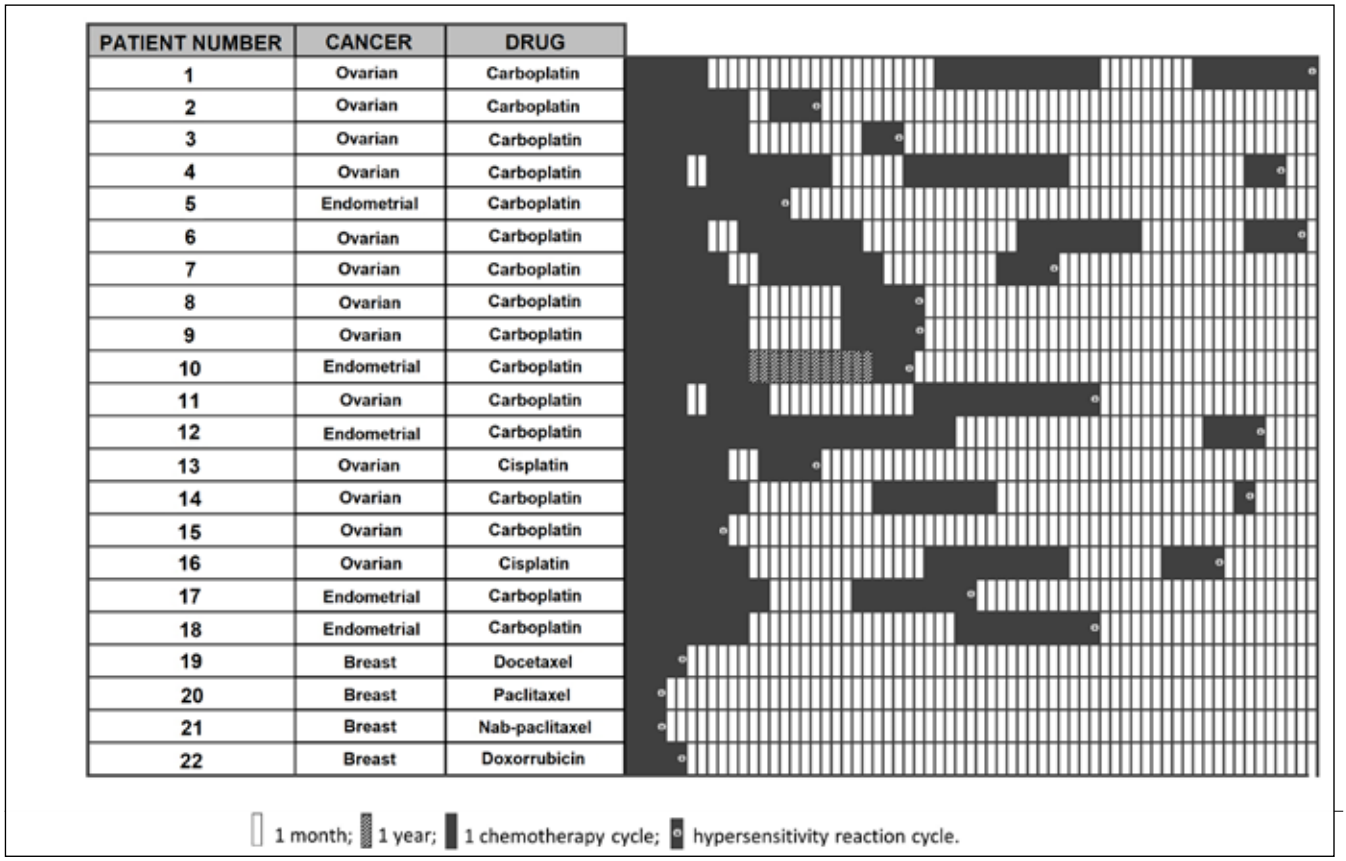
RDD was performed in all patients with the respective drug involved in the HSR, after workup through skin tests (**table I**). The median number of RDD cycles was 3.5 cycles (minimum 1 cycle; maximum 24 cycles). Complications during RDD occurred in one third of the patients (n = 6 of total n = 18), almost all grade I reactions, namely pruritus, facial flush and urticaria. In one case (patient number 14, **table I**), a severe type III reaction occurred, corresponding to anaphylactic shock during infusion of the eleventh step (third bag – highest concentration). In this case, intramuscular epinephrine, intramuscular clemastine, intravenous methylprednisolone and inhaled beta2 agonists were required. This patient had a type III reaction as inaugural HSR and positive SPT for carboplatin. The following desensitization protocol was adjusted to 15 steps, with only a type I reaction registered.

Table I *- Patient characteristics.*

Patient	Age of rdd	Obesity	Atopy	Familiar atopy	Cancer	Drug	Skin tests	HSR cycle	HSR cycle ≥ 7	BGS	Platinum dosis (mg)	Eosinophils (cells/mL)	RDD cycles	RDD complications	Complete scheme	Mortality
1	22	Yes	Yes	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin positive (Prick 1:1)	18	Yes	III	850	100	4	Yes (I)	Yes	Yes
2	50	No	No	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin negative	8	Yes	III	650	100	6	No	Yes	Yes
3	33	No	No	No	Ovarian	Carboplatin	**Positive** (ID 1:10) Cisplatin negative	8	Yes	I	645	30	2	No	Yes	No
4	65	No	No	No	Ovarian	Carboplatin	**Positive** (ID 1:100) Cisplatin negative	19	Yes	III	600	100	7	No	Yes	Yes
5	68	Yes	Yes	Yes	Endometrial	Carboplatin	**Positive** (ID 1:100) Cisplatin negative	8	Yes	III	600	0	2	Yes (I)	Yes	No
6	73	Yes	No	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin negative	19	Yes	I	550	300	24	Yes (I)	Yes	Yes
7	54	No	No	No	Ovarian	Carboplatin	**Negative** (Carboplatin and cisplatin)	14	Yes	III	500	0	3	Yes (I)	Yes	Yes
8	56	Yes	No	No	Ovarian	Carboplatin	**Positive** (ID 1:100) Cisplatin negative	10	Yes	III	500	0	4	No	Yes	Yes
9	44	No	No	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin positive (Prick 1:1)	10	Yes	I	500	200	3	No	Yes	Yes
10	77	No	No	No	Endometrial	Carboplatin	**Positive** (Prick 1:1) Cisplatin negative	8	Yes	I	460	0	5	No	Yes	No
11	50	Yes	Yes	No	Ovarian	Carboplatin	**Negative** (Carboplatin and cisplatin)	15	Yes	III	425	300	3	Yes (I)	Yes	No
12	73	No	No	No	Endometrial	Carboplatin	**Positive** (ID 1:10) Cisplatin negative	19	Yes	II	315	140	1	No	Yes	No
13	43	No	Yes	Yes	Ovarian	Cisplatin	**Positive** (ID 1:10) Carboplatin positive (ID 1:10)	8	Yes	III	130	100	6	No	Yes	Yes
14	49	No	No	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin negative	13	Yes	III	700	100	6	Yes (III)	Yes	Yes
15	41	No	No	No	Ovarian	Carboplatin	**Positive** (Prick 1:1) Cisplatin negative	5	No	III	550	100	3	No	Yes	Yes
16	68	No	No	No	Ovarian	Cisplatin	**Positive** (ID 1:10) Carboplatin positive (ID 1:10)	16	Yes	III	430	100	6	No	Yes	No
17	70	No	No	No	Endometrial	Carboplatin	**Positive** (ID 1:10) Cisplatin negative	13	Yes	III	685	0	2	No	Yes	No
18	70	Yes	No	No	Endometrial	Carboplatin	**Positive** (ID 1:10) Cisplatin negative	13	Yes	I	400	100	3	Yes (I)	Yes	No
19	53	No	Yes	No	Breast	Docetaxel	**Positive** (ID 1:10)	3	No	III	N/A	200	4	No	Yes	No
20	53	Yes	No	No	Breast	Paclitaxel	**Negative**	2	No	III	N/A	0	5	No	Yes	No
21	66	No	Yes	No	Breast	Nab-paclitaxel	**Negative**	2	No	III	N/A	0	3	No	Yes	No
22	65	No	No	No	Breast	PLD	N/A	3	No	III	N/A	0	5	No	Yes	No

BGS: Brown's grading system; HSR: hypersensitivity reactions; ID: Intradermal tests; PLD: Pegylated liposomal doxorubicin; N/A: not applicable; RDD: Rapid drug desensitization.

Figure 1 *- Time intervals between groups of cycles, according to patient and drug. The patient number is matching with table I.*



PATIENT NUMBER	CANCER	DRUG
1	Ovarian	Carboplatin
2	Ovarian	Carboplatin
3	Ovarian	Carboplatin
4	Ovarian	Carboplatin
5	Endometrial	Carboplatin
6	Ovarian	Carboplatin
7	Ovarian	Carboplatin
8	Ovarian	Carboplatin
9	Ovarian	Carboplatin
10	Endometrial	Carboplatin
11	Ovarian	Carboplatin
12	Endometrial	Carboplatin
13	Ovarian	Cisplatin
14	Ovarian	Carboplatin
15	Ovarian	Carboplatin
16	Ovarian	Cisplatin
17	Endometrial	Carboplatin
18	Endometrial	Carboplatin
19	Breast	Docetaxel
20	Breast	Paclitaxel
21	Breast	Nab-paclitaxel
22	Breast	Doxorrubicin

Figure 2 *- Signs and symptoms of chemotherapy hypersensitivity reactions according drug class.*

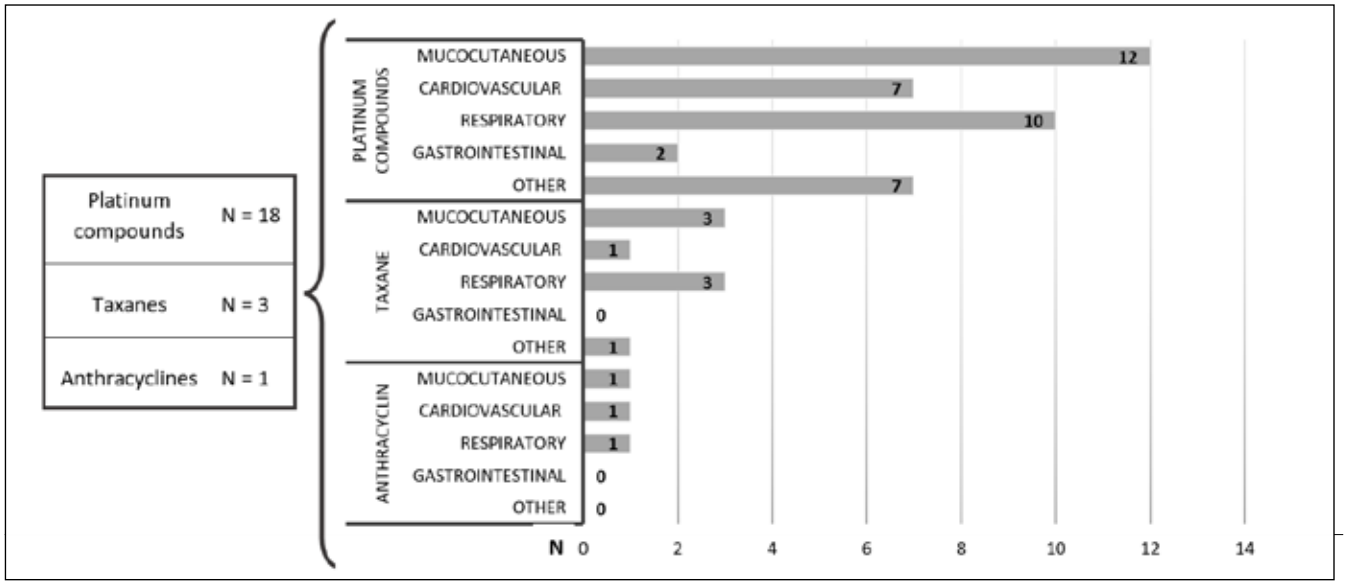


Table II *- Characterization of types and subtypes of primary cancer organ. The patient number is matching with table I.*

Patient Number	Type of gynecological cancer	Age at diagnosis	BRCA mutation/ Lynch syndrome	FIGO staging	Distant metastasis at diagnosis	Disease progression	Age of death
	Ovarian cancer						
9	High-grade serous	41	No	IIIC	No	Yes	44
6	High-grade serous	66	No	IIIC	No	Yes	78
11	High-grade serous	47	No	IIIB	No	Yes	N/A
2	High-grade serous	47	No	IIA	No	Yes	51
16	High-grade serous	65	BRCA 1	IIIA1	No	No	N/A
13	High-grade serous	42	No	IIC	No	Yes	45
7	High-grade serous	49	No	IIIC	No	Yes	55
8	High-grade serous	53	No	IVA	Pleura	Yes	64
14	High-grade serous	44	No	IIIA1	No	Yes	54
3	Low-grade serous	30	VUS in BRCA 2	IIC	No	Yes	N/A
4	Clear-cell	61	No	IIB	No	Yes	68
15	Clear-cell	38	No	IIIC	No	Yes	42
1	Juvenile granulosa cell tumour	22	No	IC2	No	Yes	32
	Endometrial cancer						
18	Endometrioid grade 2	64	No	IIIB	No	Yes	N/A
12	Endometrioid grade 3	69	No	IVA	No	Yes	N/A
17	Endometrioid grade 3	66	No	IVB	Lung	Yes	N/A
10	Serous	65	No	IIIA	No	Yes	N/A
5	Clear-cell	67	No	IVB	Gastric	Yes	N/A
	Breast cancer			**AJCC staging**			
22	Invasive carcinoma NOS (Luminal B like - HER2 negative)	45	No	IA	No	Yes	N/A
21	Invasive carcinoma NOS (Luminal B like - HER2 negative)	51	No	IV	Liver, Pleura, Bone	Yes	N/A
20	Invasive carcinoma NOS (Triple-negative)	48	No	IIIB	No	Yes	N/A
19	Invasive carcinoma NOS (Luminal B like - HER2 negative)	53	No	IV	Lung, Bone	Yes	N/A

AJCC: American Joint Committee on Cancer; BRCA: Breast Cancer gene; FIGO: International Federation of Gynecology and Obstetrics; N/A: not applicable; NOS: not otherwise specified; HER2: Epidermal growth factor receptor 2; VUS: variant of unknown significance.

All patients completed the proposed chemotherapy desensitization protocol.

Taxanes: this was the second most frequent group of chemotherapy drugs that resulted in HSRs and RDD in our sample, corresponding to a total of 13.6% (n = 3) of patients (n = 1 to docetaxel, n = 1 to nab-paclitaxel and n = 1 to paclitaxel). The three patients were initially diagnosed with advanced breast cancer by 2015, resulting in a 5-year survival rate of 100.0%. Regarding comorbidities, two patients presented atopy and one patient was obese.

HSRs in this group occurred at an early stage of the chemotherapy regimen (minimum 2, maximum 3 cycles), and all patients had a grade III HSR.

Considering the patient with HSR to docetaxel, SPT were positive for ID 1:10. The patients with paclitaxel and nab-paclitaxel HSRs had negative SPT for the respective drug.

The median number of RDD cycles was 4.0 (minimum 3 cycles, maximum 5 cycles) and none of the patients experienced complications during the RDD cycles.

All patients completed the proposed chemotherapy desensitization protocol.

Anthracyclines: only one patient suffered from HSR (patient number 22, **table II**) to PLD and underwent RDD. The patient had a diagnosis of non-metastatic breast cancer and presented no other diseases/comorbidities. HSR occurred in the 3rd cycle and was characterized as severe (grade III). No skin tests were performed. The patient underwent 5 cycles of RDD without complications, and has a current disease-free survival of 3 years.

Discussion

In this study we report the experience of our hospital in 107 successful RDD performed in 22 patients who experienced immediate type HSRs to platinum compounds, taxanes, and anthracyclines. Only one patient experienced a severe complication (HSR grade III) during the first 12-step RDD, which was subsequently changed to a 15-step protocol with no complications.

It is well established that any chemotherapy agent can cause HSRs; however platinum and taxanes are the most common agents involved in HSRs and our results are in accordance with this (54). Most of our patients presented HSRs to platinum agents, and this is also partly justified by the wide use of this group in the first line of treatment of solid tumours in adults, especially for ovarian cancer.

The difference in the median time interval between the HSR and previous chemotherapy cycle in ovarian and endometrial cancer is related to the fact that there is a higher rate of relapse in the former.

In taxanes, the HSRs occurred despite Ginecology-Oncology premedication protocol, that includes: dexamethasone 10 mg and H1 and H2 antihistamines (clemastine 2 mg, ranitidine 50 mg), in the cases of paclitaxel and nab-paclitaxel (55); dexamethasone 8 mg (on the day before, the day of the treatment and the day after), in the case of docetaxel (55, 56).

The patients selected for desensitization were those who had immediate HSRs and, therefore, the mechanism likely to be implied is based on IgE-induced sensitization, although skin tests did not confirm this in all patients, as in other published series (57). In our study, the patients selected for RDD were those with a clinical history compatible with immediate HSRs and positive SPT; and in patients with negative SPT, only those diagnosed with a more severe immediate HSRs - grade III.

Premedication for platinum includes antiemetic drugs and dexamethasone, and there is no premedication prescribed in the case of PLD (55).

Most patients in the three types of cancer had an advanced stage of the disease at the time of diagnosis, which generally requires higher doses of chemotherapy, a greater number of cycles and consequently increased chances of HSRs, and these are well established risk factors for HSRs to platinum compounds (32).

Taking into account previous investigations in which it was reported that a drug allergy history correlated with predictive risk of chemotherapy allergy (32, 58), especially with platinum compounds, our sample showed a low prevalence, not only regarding previous drug allergy history but also other atopic diseases, when compared to other results in the literature (5). The presence of obesity has been associated with severe manifestations and fatal outcomes in anaphylaxis (59, 60). In our sample, obesity was also present in a significant number of patients, not only in the platinum group, but also for taxanes, considering that almost half of these patients presented severe grade III HSRs. These data highlight obesity as a possible predictor of chemotherapy HSRs, although further studies are needed with a larger sample size.

The association of paclitaxel with platinum chemotherapy schemes improves overall survival in patients with recurrent platinum-sensitive ovarian cancer (5). Our work has also shown that this association was very prevalent, corroborating that the combination of these drugs is considered a risk factor (26).

Although pathogenic mutations in BRCA 1/2 appear to increase the risk of HSRs to carboplatin infusion (23), our population showed a low prevalence of this mutation which was confirmed in only one woman with a cisplatin HSR. The other woman with VUS BRCA 2 mutation had a carboplatin HSR. These patients also presented heterogeneity in HSR severity, with the pathogenic variant associated with a higher-grade reaction (III).

The relationship between the presence of eosinophils and allergic diseases is already known, and has been studied as a possible risk factor in drug HSRs, especially regarding their count in the specific platinum therapeutic cycle in which the allergic reaction occurred (61). However, some studies carried out in this area have proven an absence of relationship or even the presence of a lower number of eosinophils in patients with platinum reactions (41), as shown in our data.

In the platinum compounds HSR cases, almost the entire sample (n = 17, total n = 18) presented HSRs after ≥ 7 cycles of che-

motherapy, which is in keeping with the number of cycles being considered a risk factor for allergic reactions (32). The same is not true for the other groups of drugs studied, which is also in agreement with the literature (16, 33).

The positive skin tests did not correlate with the severity of the initial reaction, as there were cases in which the tests were positive and the HSR was mild (grade I) and cases in which the tests were negative and the HSRs were severe (grade III). Although platinum skin tests are recommended and negative test results are associated with a lower risk of anaphylaxis (45, 62), all patients in our sample with negative skin tests had severe type III reactions, so RDD was performed in accordance to the risk assessed via clinical history. Even considering that platinum skin tests are recommended and validated, and are a useful complementary diagnostic tool, this must always be associated with a complete and exhaustive clinical history to assess the risk of a future desensitization procedure.

Skin tests for paclitaxel showed negative results in all tested cases, which is in line with data presented in literature, corroborating that the predictive value has not yet been demonstrated (63).

The high mortality rate of our sample (50.0%) is justified by advanced oncological disease at the date of the first HSR to chemotherapy. The same may justify the fact that, in the case of platinum salts, in which a higher dose is associated with an increased risk of HSRs, the vast majority of patients had been submitted to multiple treatment lines.

Conclusions

In all presented cases, rapid drug desensitization successfully allowed the reintroduction of antineoplastic drugs in patients with a previous history of immediate hypersensitivity reaction. Of all patients included, only one had a serious complication (anaphylaxis) during the course of the desensitization protocol, and it was necessary to adjust the protocol from 12 to 15 steps in the following cycles.

A joint protocol between the departments of gynaecology and allergy and clinical immunology allows the patients to benefit from better clinical guidance, resulting in the conclusion of the proposed chemotherapy in cases of HSRs.

Our work demonstrated the safety and effectiveness of these protocols, highlighting the advantages of gaining experience in this procedure.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, *et al*. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Ann Oncol. 2019;30(5):672-705. doi: 10.1093/annonc/mdz062.
2. Prat J; FIGO Committee on Gynecologic Oncology. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet. 2014;124(1):1-5. doi: 10.1016/j.ijgo.2013.10.001.
3. Dinkelspiel HE, Champer M, Hou J, Tergas A, Burke WM, Huang Y, *et al*. Long-term mortality among women with epithelial ovarian cancer. Gynecol Oncol. 2015;138(2):421-8. doi: 10.1016/j.ygyno.2015.06.005.
4. LaVigne K, Hyman DM, Zhou QC, Iasonos A, Tew WP, Aghajanian C, *et al*. A Randomized Trial of Prophylactic Extended Carboplatin Infusion to Reduce Hypersensitivity Reactions in Recurrent Ovarian Cancer. Int J Gynecol Cancer. 2018;28(6):1176-82. doi: 10.1097/IGC.0000000000001280.
5. Tai YH, Tai YJ, Hsu HC, Lee SP, Chen YY, Chiang YC, *et al*. Risk Factors of Hypersensitivity to Carboplatin in Patients with Gynecologic Malignancies. Front Pharmacol. 2017;8:800. doi: 10.3389/fphar.2017.00800.
6. Global Cancer Observatory (Globocan). Available at: https://gco.iarc.fr/. Last access date: 19/06/2020.
7. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, *et al*. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. Ann Oncol. 2016;27(1):16-41. doi: 10.1093/annonc/mdv484.
8. NIH - National Cancer Institute. Avialble at: https://www.cancer.gov/. Last access date: 19/06/2020.
9. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours of Female Reproductive Organs. IARC, 2014, Lyon.
10. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4. doi: 10.1016/j.ijgo.2009.02.012.
11. Querleu D, Darai E, Lecuru F, Rafii A, Chereau E, Collinet P, *et al*. Prise en charge primaire des cancers de l'endomètre : recommandations SFOG-CNGOF [Primary management of endometrial carcinoma. Joint recommendations of the French society of gynecologic oncology (SFOG) and of the French college of obstetricians and gynecologists (CNGOF)]. Gynecol Obstet Fertil Senol. 2017;45(12):715-725. French. doi: 10.1016/j.gofs.2017.10.008.
12. de Boer SM, Powell ME, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, *et al*. Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial. Lancet Oncol. 2019;20(9):1273-85. doi: 10.1016/S1470-2045(19)30395-X.
13. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, *et al*. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019;30(8):1194-220. doi: 10.1093/annonc/mdz173.
14. Jensen-Jarolim E, Bax HJ, Bianchini R, Capron M, Corrigan C, Castells M, *et al*. AllergoOncology - the impact of allergy in oncology: EAACI position paper. Allergy. 2017;72(6):866-87. doi: 10.1111/all.13119.
15. Cernadas JR, Brockow K, Romano A, Aberer W, Torres MJ, Bircher A, *et al*. General considerations on rapid desensitization for drug hypersensitivity - a consensus statement. Allergy. 2010;65(11):1357-66. doi: 10.1111/j.1398-9995.2010.02441.x.
16. Syrigou E, Triantafyllou O, Makrilia N, Kaklamanos I, Kotanidou A, Manolopoulos L, *et al*. Acute hypersensitivity reactions to chemotherapy agents: an overview. Inflamm Allergy Drug Targets. 2010;9(3):206-13. doi: 10.2174/187152810792231887.

17. Scherer K, Brockow K, Aberer W, Gooi JH, Demoly P, Romano A, *et al*. Desensitization in delayed drug hypersensitivity reactions -- an EAACI position paper of the Drug Allergy Interest Group. Allergy. 2013;68(7):844-52. doi: 10.1111/all.12161.
18. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA, *et al*. International Consensus on drug allergy. Allergy. 2014;69(4):420-37. doi: 10.1111/all.12350.
19. Castells M. Drug Hypersensitivity and Anaphylaxis in Cancer and Chronic Inflammatory Diseases: The Role of Desensitizations. Front Immunol. 2017;8:1472. doi: 10.3389/fimmu.2017.01472.
20. Li Q, Cohn D, Waller A, Backes F, Copeland L, Fowler J, *et al*. Outpatient rapid 4-step desensitization for gynecologic oncology patients with mild to low-risk, moderate hypersensitivity reactions to carboplatin/cisplatin. Gynecol Oncol. 2014;135(1):90-4. doi: 10.1016/j.ygyno.2014.07.104.
21. Chung SJ, Kang SY, Kang RY, Kim YC, Lee KH, Kim TY, *et al*. A new non-dilution rapid desensitization protocol successfully applied to all-grade platinum hypersensitivity. Cancer Chemother Pharmacol. 2018 Nov;82(5):777-785. doi: 10.1007/s00280-018-3662-0.
22. Hesterberg PE, Banerji A, Oren E, Penson RT, Krasner CN, Seiden MV, *et al*. Risk stratification for desensitization of patients with carboplatin hypersensitivity: clinical presentation and management. J Allergy Clin Immunol. 2009;123(6):1262-7.e1. doi: 10.1016/j.jaci.2009.02.042.
23. Otani IM, Wong J, Banerji A. Platinum Chemotherapy Hypersensitivity: Prevalence and Management. Immunol Allergy Clin North Am. 2017;37(4):663-77. doi: 10.1016/j.iac.2017.06.003.
24. Abe A, Ikawa H, Ikawa S. Desensitization treatment with cisplatin after carboplatin hypersensitivity reaction in gynecologic cancer. J Med Invest. 2010;57(1-2):163-7. doi: 10.2152/jmi.57.163.
25. Rogers BB, Cuddahy T, Briscella C, Ross N, Olszanski AJ, Denlinger CS. Oxaliplatin: Detection and Management of Hypersensitivity Reactions. Clin J Oncol Nurs. 2019;23(1):68-75. doi: 10.1188/19.CJON.68-75.
26. Miyamoto S, Okada R, Ando K. Platinum hypersensitivity and desensitization. Jpn J Clin Oncol. 2015;45(9):795-804. doi: 10.1093/jjco/hyv081.
27. Castells MC. Anaphylaxis to chemotherapy and monoclonal antibodies. Immunol Allergy Clin North Am. 2015;35(2):335-48. doi: 10.1016/j.iac.2015.01.011.
28. Saunders MP, Denton CP, O'Brien ME, Blake P, Gore M, Wiltshaw E. Hypersensitivity reactions to cisplatin and carboplatin--a report on six cases. Ann Oncol. 1992;3(7):574-6. doi: 10.1093/oxfordjournals.annonc.a058265.
29. Mezzano V, Giavina-Bianchi P, Picard M, Caiado J, Castells M. Drug desensitization in the management of hypersensitivity reactions to monoclonal antibodies and chemotherapy. BioDrugs. 2014;28(2):133-44. doi: 10.1007/s40259-013-0066-x.
30. Parel M, Ranchon F, Nosbaum A, You B, Vantard N, Schwiertz V, *et al*. Hypersensitivity to oxaliplatin: clinical features and risk factors. BMC Pharmacol Toxicol. 2014;15:1. doi: 10.1186/2050-6511-15-1.
31. Callahan MB, Lachance JA, Stone RL, Kelsey J, Rice LW, Jazaeri AA. Use of cisplatin without desensitization after carboplatin hypersensitivity reaction in epithelial ovarian and primary peritoneal cancer. Am J Obstet Gynecol. 2007;197(2):199.e1-4; discussion 199.e4-5. doi: 10.1016/j.ajog.2007.04.044.
32. Picard M, Matulonis UA, Castells M. Chemotherapy hypersensitivity reactions in ovarian cancer. J Natl Compr Canc Netw. 2014;12(3):389-402. doi: 10.6004/jnccn.2014.0040.
33. Picard M. Management of Hypersensitivity Reactions to Taxanes. Immunol Allergy Clin North Am. 2017;37(4):679-93. doi: 10.1016/j.iac.2017.07.004.
34. Giavina-Bianchi P, Patil SU, Banerji A. Immediate Hypersensitivity Reaction to Chemotherapeutic Agents. J Allergy Clin Immunol Pract. 2017;5(3):593-9. doi: 10.1016/j.jaip.2017.03.015.
35. 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.
36. Bonamichi-Santos R, Castells M. Diagnoses and Management of Drug Hypersensitivity and Anaphylaxis in Cancer and Chronic Inflammatory Diseases: Reactions to Taxanes and Monoclonal Antibodies. Clin Rev Allergy Immunol. 2018;54(3):375-85. doi: 10.1007/s12016-016-8556-5.
37. Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique NM, Alving CR, *et al*. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. Ann Oncol. 2003;14(9):1430-7. doi: 10.1093/annonc/mdg374.
38. Joly F, Ray-Coquard I, Fabbro M, Donoghoe M, Boman K, Sugimoto A, *et al*. Decreased hypersensitivity reactions with carboplatin-pegylated liposomal doxorubicin compared to carboplatin-paclitaxel combination: analysis from the GCIG CALYPSO relapsing ovarian cancer trial. Gynecol Oncol. 2011;122(2):226-32. doi: 10.1016/j.ygyno.2011.04.019.
39. Aoyama T, Takano M, Miyamoto M, Yoshikawa T, Soyama H, Kato K, *et al*. Is there any predictor for hypersensitivity reactions in gynecologic cancer patients treated with paclitaxel-based therapy? Cancer Chemother Pharmacol. 2017;80(1):65-9. doi: 10.1007/s00280-017-3332-7.
40. Sendo T, Sakai N, Itoh Y, Ikesue H, Kobayashi H, Hirakawa T, *et al*. Incidence and risk factors for paclitaxel hypersensitivity during ovarian cancer chemotherapy. Cancer Chemother Pharmacol. 2005;56(1):91-6. doi: 10.1007/s00280-004-0924-9.
41. Makrilia N, Syrigou E, Kaklamanos I, Manolopoulos L, Saif MW. Hypersensitivity reactions associated with platinum antineoplastic agents: a systematic review. Met Based Drugs. 2010;2010:207084. doi: 10.1155/2010/207084.
42. Castells M. Rapid desensitization for hypersensitivity reactions to medications. Immunol Allergy Clin North Am. 2009;29(3):585-606. doi: 10.1016/j.iac.2009.04.012.
43. Castells M. Rapid desensitization for hypersensitivity reactions to chemotherapy agents. Curr Opin Allergy Clin Immunol. 2006;6(4):271-7. doi: 10.1097/01.all.0000235900.57182.15.
44. Lee CW, Matulonis UA, Castells MC. Carboplatin hypersensitivity: a 6-h 12-step protocol effective in 35 desensitizations in patients with gynecological malignancies and mast cell/IgE-mediated reactions. Gynecol Oncol. 2004;95(2):370-6. doi: 10.1016/j.ygyno.2004.08.002.
45. 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.
46. 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.
47. Pichler WJ (ed): Drug Hypersensitivity. Basel, Karger, 2007: pp 413-25. doi: 10.1159/000104218
48. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, *et al*. The 2019 World Health Organization classification of tumours of the breast. Histopathology. 2020;77(2):181-5. doi: 10.1111/his.14091.

49. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, *et al*. 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.
50. 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.
51. 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):990-7. doi: 10.1016/j.jaip.2018.09.018.
52. Castells Guitart MC. Rapid drug desensitization for hypersensitivity reactions to chemotherapy and monoclonal antibodies in the 21st century. J Investig Allergol Clin Immunol. 2014;24(2):72-9; quiz 2 p following 79. Available at: https://pubmed.ncbi.nlm.nih.gov/24834769/.
53. Burches E, Pérez-Fidalgo JA, Ferriols F, González-Barrallo I, Cervantes A. A logarithmic rapid desensitization protocol: initial experience in carboplatin hypersensitivity reactions. J Cancer Metastasis Treat. 2019;5:63. doi: 10.20517/2394-4722.2019.012.
54. Pagani M. The complex clinical picture of presumably allergic side effects to cytostatic drugs: symptoms, pathomechanism, reexposure, and desensitization. Med Clin North Am. 2010;94(4):835-52, xiii. doi: 10.1016/j.mcna.2010.03.002.
55. eviQ and Cancer Institute. Available at: https://www.eviq.org.au/pages/about-us. Last access date: 19/06/2020.
56. Picard M, Pur L, Caiado J, Giavina-Bianchi P, Galvão VR, Berlin ST, *et al*. Risk stratification and skin testing to guide re-exposure in taxane-induced hypersensitivity reactions. J Allergy Clin Immunol. 2016 Apr;137(4):1154-1164.e12. doi: 10.1016/j.jaci.2015.10.039.
57. Caiado J, Rodrigues T, Pedro E, Costa L, Barbosa MP. Dessensibilização a fármacos em oncologia: Experiência de um Serviço de Imunoalergologia. Rev Port Imunoalergologia. 2009;17:57-74. Available at: https://www.spaic.pt/client_files/rpia_artigos/dessensibilizacao-a-farmacos-em-oncologia:-experiencia-de-um-servico-de-imunoalergologia.pdf.
58. Libra M, Sorio R, Buonadonna A, Berretta M, Stefanovski P, Toffoli G, *et al*. Cisplatin may be a valid alternative approach in ovarian carcinoma with carboplatin hypersensitivity. Report of three cases. Tumori. 2003;89(3):311-3. Avaiable at: https://pubmed.ncbi.nlm.nih.gov/12908789/.
59. Turner PJ, Jerschow E, Umasunthar T, Lin R, Campbell DE, Boyle RJ. Fatal Anaphylaxis: Mortality Rate and Risk Factors. J Allergy Clin Immunol Pract. 2017;5(5):1169-78. doi: 10.1016/j.jaip.2017.06.031.
60. Alen Coutinho I, Ferreira D, Regateiro FS, Pita J, Ferreira M, Martins JF, *et al*. Anaphylaxis in an emergency department: a retrospective 10-year study in a tertiary hospital. Eur Ann Allergy Clin Immunol. 2020;52(1):23-34. doi: 10.23822/EurAnnACI.1764-1489.98.
61. Cetean S, Ciuleanu T, Leucuta DC, Cainap C, Constantin AM, Cazacu I, *et al*. Hypersensitivity reactions to platinum derivatives: findings of new predictive markers. J BUON. 2015;20(6):1617-23. Available at: https://pubmed.ncbi.nlm.nih.gov/26854461/.
62. 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.
63. Lagopoulos V, Gigi E. Anaphylactic and anaphylactoid reactions during the perioperative period. Hippokratia. 2011;15(2):138-40. Available at: https://pubmed.ncbi.nlm.nih.gov/22110295/.

M. C. Sánchez-Hernández[1], M. T. Dordal[2], A. M. Navarro[1], I. Dávila[3], B. Fernández-Parra[4], C. Colás[5], C. Rondón[6], A. del Cuvillo[7], F. Vega[8], J. Montoro[9], M. Lluch-Bernal[10], V. Matheu[11], P. Campo[6], M. L. González[12], R. González-Pérez[11], A. Izquierdo-Domínguez[13], A. Puiggros[14], M. Velasco[15], A. Fernández-Palacín[16], A. Valero[17], SEAIC Rhinoconjunctivitis Committee 2014-2018

Severity and duration of allergic conjunctivitis: are they associated with severity and duration of allergic rhinitis and asthma?

[1]Allergology UGS, Hospital Universitario Virgen Macarena, Sevilla, Spain
[2]Allergy Unit, Department of Internal Medicine, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain
[3]Allergy Service, Hospital Universitario de Salamanca, Salamanca, Spain
[4]Department of Allergology, Hospital El Bierzo, Ponferrada, León, Spain
[5]Department of Allergology, Hospital Clínico Universitario-Instituto de Investigación Sanitaria de Aragón, Universidad de Zaragoza, Zaragoza, Spain
[6]Allergy Unit, Hospital Universitario Regional de Málaga-ARADyAL, UMA, Málaga, Spain
[7]Asthma and Rhinitis Unit, Department of Otorhinolaryngology, Hospital de Jerez, Jerez, Spain
[8]Department of Allergology, Hospital de la Princesa, Instituto de Investigación Sanitaria (IP), Madrid, Spain
[9]Allergy Unit, Faculty of Medicine, Universidad Católica de Valencia San Vicente Mártir, Hospital de Líria, Valencia, Spain
[10]Department of Allergology, Hospital La Paz, Madrid, Spain
[11]Allergy Service, Hospital Universitario de Canarias, Tenerife, Spain
[12]Department of Allergology, Hospital Clínico San Carlos, Madrid, Spain
[13]Allergy Service, Consorci Sanitari of Terrassa, and Allergy Unit, Clinica Diagonal, Barcelona, Spain
[14]Allergy Unit, Hospital Quirón, Barcelona, Spain
[15]Allergy Unit, CCEE Araba, Vitoria, Spain
[16]Biostatistics Unit, Department of Preventive Medicine and Public Health, Universidad de Sevilla, Sevilla, Spain
[17]Department of Pneumology and Allergy, Hospital Clínic i Universitari, Institut d´Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), CIBERES, Barcelona, Spain

Key words

Allergic conjunctivitis; allergic comorbidity; allergic respiratory disease; epidemiology; one airway.

Corresponding author
María Cesárea Sánchez-Hernández
Allergology UGS
Hospital Universitario Virgen Macarena
C/ Dr. Fedriani 3
41009 Sevilla, Spain
ORCID ID: 0000-0002-0157-1161
E-mail: mcesar.sanchez@hotmail.es

Doi
10.23822/EurAnnACI.1764-1489.231

Impact statement

The greater the severity and duration of conjunctivitis, the greater the severity and duration of rhinitis and asthma.

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

Summary

Objective. *The association of allergic conjunctivitis (AC) with rhinitis and/or asthma is poorly understood. The objective of this study was to apply the Consensus Document for Allergic Conjunctivitis (DECA) criteria for the classification of AC to a population of patients with AC to assess the association between the severity and duration of AC and rhinitis and/or asthma.* ***Methods.*** *Patients with ocular symptoms of AC who participated in the "Alergológica 2015" study were included. The demographics, classification according to the DECA criteria, etiology, and comorbidities were evaluated by age groups (≤ 14 and > 14 years).* ***Results.*** *A total of 2,914 patients (age range, 1-90 years) were included in the "Alergológica 2015" study. Of these, 965 patients (33.1%) were diagnosed with AC (77.5% > 14 years). AC was classified as severe, moderate, or mild in 1.8%, 46.4%, and 51.8%, respectively; and as intermittent or persistent in 51.6% and 48.4% of the patients. AC alone occurred in 4% of patients. AC was mainly associated with rhinitis (88.4%), asthma (38.2%), food allergy (8.3%) and atopic dermatitis (3.5%). In allergic respiratory disease rhinitis preceded AC and asthma developed later. The severity and duration of AC was significantly associated with severity and duration of rhinitis ($p < 0.001$ for both age groups) and asthma ($p < 0.001$ only in adults).* ***Conclusions.*** *The application of the new DECA classification for AC reveals a direct relationship between AC, rhinitis and asthma respect to severity and duration. These relationships suggest that AC should be considered an integral part of the "one airway, one disease' hypothesis.*

Introduction

Allergic conjunctivitis (AC) is an inflammatory reaction of the conjunctiva mediated by immunoglobulin (Ig)E hypersensitivity. Depending on the geographical area and study design, the estimated prevalence of AC in the general population ranges between 15 and 40% (1-3).

AC is often associated with other atopic conditions such as eczema, food allergy, and especially rhinitis and asthma (4). Since the allergic response often involves the conjunctival surface of the eye as well as the respiratory tract, it has been hypothesized that AC should be considered as part of the "*united airway disease", a concept based on the anatomical and functional* links between the upper and the lower respiratory tracts (5-7). Likewise, the concept of allergic respiratory disease (ARD), based on the allergic origin of the disease and its clinical spectrum, includes conjunctivitis, rhinitis, and/or asthma, although not all clinical manifestations must occur simultaneously in patients with ARD (8).

The classification of allergic rhinitis and asthma according to duration and severity has made it possible to demonstrate a strong association between both entities (9). For example, it has been observed that the greater the severity and duration of rhinitis, the greater the possibility of being associated with asthma (10). However, the role of AC as a risk factor of rhinitis and/or asthma is poorly understood, possibly due to the lack of a validated classification based on the duration and severity of the disease. We have recently validated the new criteria for classification of AC's severity and control proposed in the Consensus Document on Allergic Conjunctivitis (DECA) (11, 12). The present study aimed to apply the DECA classification to assess the association between the severity and duration of AC and its main comorbidities, rhinitis, and asthma, for the first time.

Materials and methods

Study design and population

The "Alergológica 2015" study was a multicenter, observational, cross-sectional, prospective study of patients consulting an allergist for the first time in public and private health centers in Spain, between March 2014 and March 2015, whose material and methods have been published elsewhere (13-15). The "Alergológica 2015" study was approved by the Clinical Research Ethics Committee of Hospital General de la Defensa, Madrid, Spain. Patients or legal guardians signed written informed consent. In the "Alergológica 2015" study data were collected on an electronic case report form (CRF). The CRF remained open until the diagnostic work-up had been completed for all patients or until the end of the recruitment period. Clinical symptoms, time from the onset of disease to the study inclusion, demographic data (age, gender, and habitat), smoking behavior, and family history of allergic diseases were recorded. Complementary diagnostic tests were performed following the investigator's criteria and consisted of skin tests, specific IgE determinations, functional respiratory tests, provocation tests, and others, following standard clinical practice.

In the present study, adults and children with suggestive AC symptoms fulfilling DECA criteria were retrospectively analyzed (**table I**) (11). The demographics, skin prick test, classification according to the DECA criteria, and comorbidities, were evaluated by age groups (≤ 14 and > 14 years). AC, rhinitis and asthma were classified, respectively, according to DECA criteria (**figure 1**) (11), modified Allergic Rhinitis and its Impact on Asthma (ARIA) criteria (16), and Spanish guide for management of asthma (GEMA) criteria (17).

Table I *- Clinical criteria for suspicion of allergic conjunctivitis (DECA) (11).*

Bilateral conjunctival hyperemia and pruritus (together with at least 3 of the following criteria)
1. Ocular symptoms associated with exposure to suspicious allergens
2. Association with other allergic diseases (rhinitis, asthma, atopic dermatitis)
3. Response to topical pharmacologic therapy (antihistamines, mast cell stabilizers, dual-action agents)
4. Absence of giant papillary conjunctivitis
5. Absence of corneal involvement

Statistical analysis

Data were expressed as mean ± standard deviation (SD). When numeric distribution was markedly asymmetric, median and percentiles (P 25 and P 75) were used. Qualitative variables were calculated based on frequencies and percentages. Data were analyzed both globally and by age groups: ≤ 14 years old (pediatric group) and > 14 years old (adults' group). The relationship between qualitative variables was analyzed using the Chi-square test, Chi-square with correction for continuity, or Fisher's exact test. The Chi-squared test was used to analyze relationships between two categorical variables. For the study of concordance, Cohen's Kappa coefficients were obtained for qualitative variables. Agreement was considered fair if the value of Kappa was 0.21-0.40, moderate if 0.41-0.60, substantial if 0.61-0.80, and almost perfect if 0.81-1.00 (18). A statistical significance level of 0.05 was considered for all tests. The software IBM SPSS Statistics v25 for Windows (Armonk, NY, USA) was used for the statistical analysis.

Results

Demographics

Of the 2,914 patients included in the "Alergológica 2015" study, 965 patients (33.1%) were diagnosed with AC. Of the patients with AC (age range, 1-90 years), 17.3% were aged ≤ 14 years (**table II**). In the pediatric group, there was male predominance (62.0%, $p < 0.001$), but in the adult population, most patients were female (57.4%).

Globally, 66.0% of patients with AC referred family history of atopy, rhinitis (52.0%), asthma (29.5%), and conjunctivitis (23.4%). Most of the patients lived in urban areas (62%) and were non-smokers (74%). Exposure to pets was referred by 15.5% of patients.

Skin prick tests

The pollens were the most frequent allergen detected by skin tests in the population, both children and adults (**table II**). Grass pollen was the most frequent sensitization (49%), followed by *Olea europaea* (37%). Among the mites, the most prevalent were *Dermatophagoides pteronyssinus* (27.9%), *D. farinae* (20.8%), and *Lepidoglyphus destructor* (6.5%). The most prevalent mold was *Alternaria alternata* (4.5 %). Cats (12%) and dogs (8.5%) were the most frequent sensitizations to animals. Sensitization to hamsters was related to pet ownership ($p < 0.01$).

Figure 1 *- Criteria for the Classification of AC according to the DECA criteria (11).*

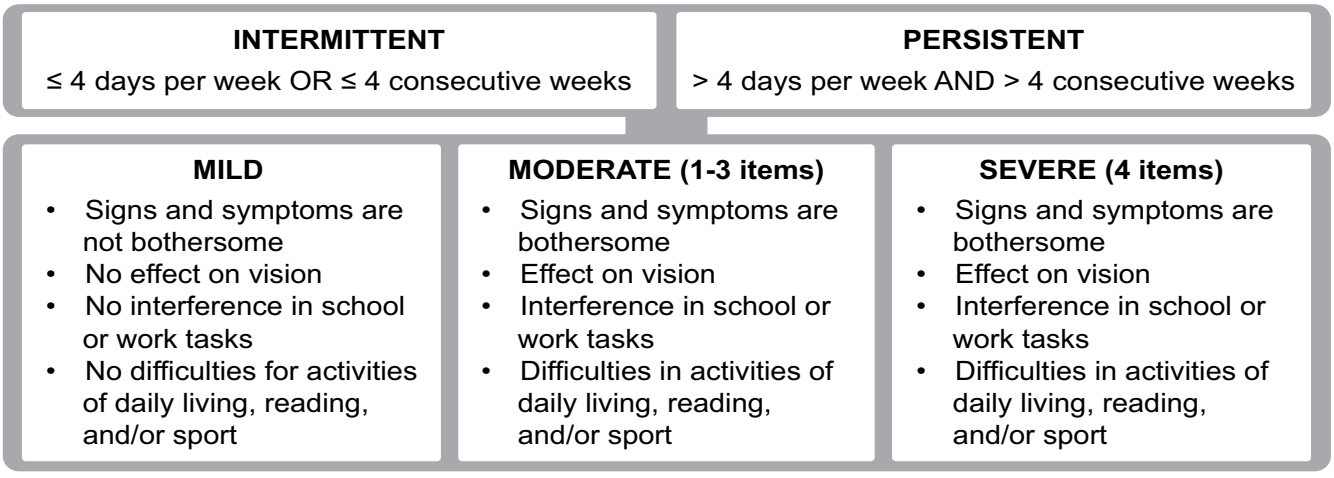


Table II *- Demographic and clinical characteristics of the patients with confirmed AC included in the "Alergológica 2015" study.*

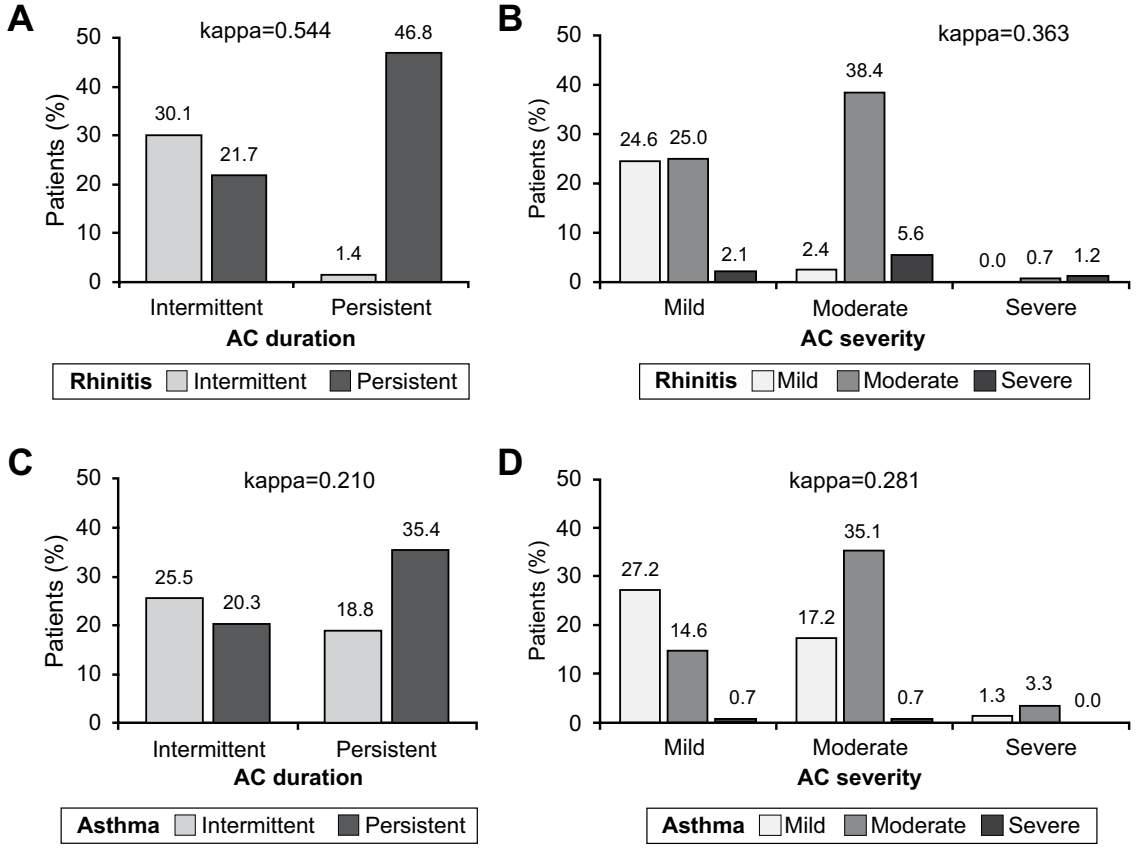
Variable	≤ 14 years (N = 167)	> 14 years (N = 748)	Total (N = 965)
Gender (female), N (%)	N = 163	N = 734	N = 897
Female	62 (38.0)	422 (57.4)	484 (54.0)
Male	101 (62.0)*	312 (42.6)	413 (46.0)
Allergen, N (%)	N = 167	N = 748	N = 915
Pollens	102 (61.1)	490 (65.5)	592 (64.7)
Mites	53 (31.7)	217 (29.0)	270 (29.5)
Animal dander	17 (10.2)	125 (16.7)**	142 (15.5)
Molds	13 (7.8)**	29 (3.9)	42 (4.6)
AC classification, N (%)	N = 156	N = 683	N = 839
Intermittent mild	57 (36.5)	242 (35.4)	299 (35.6)
Intermittent moderate	21 (13.5)	111 (16.3)	132 (15.7)
Intermittent severe	0 (0.0)	2 (0.3)	2 (0.2)
Persistent mild	25 (16.0)	111 (16.3)	136 (16.2)
Persistent moderate	50 (32.1)	207 (30.3)	257 (30.6)
Persistent severe	3 (1.9)	10 (1.5)	13 (1.5)
Comorbidities, N (%)			
Allergic rhinitis	155 (92.8)	698 (81.8)	853 (88.4)
Intermittent mild	27 (17.4)	109 (15.6)	136 (15.9)
Intermittent moderate	21 (13.5)	120 (17.2)	141 (16.5)
Intermittent severe	1 (0.6)	1 (0.1)	2 (0.2)
Persistent mild	24 (15.5)	73 (10.5)	97 (11.4)
Persistent moderate	75 (48.4)	331 (47.4)	406 (47.6)
Persistent severe	7 (4.5)	64 (9.2)	71 (8.3)
Allergic asthma	79 (47.3)	290 (38.8)	369 (38.2)
Occasional episodic/intermittent	36 (45.5)	127 (44)	-
Frequent episodic	21 (26.6)	-	-
Persistent mild	-	78 (27)	-
Persistent moderate	22(27.8)	83 (29)	-
Persistent severe	-	2 (0.7)	-
Food allergy	9 (5.3)	65 (8.6)	80 (8.3)
Atopic dermatitis	16 (9.5)*	15 (2.0)	34 (3.5)

*p < 0.05; **p < 0.001.

AC classification

All patients were classified according to duration and severity using the validated DECA classification (**table II**). The severity of the AC symptoms was mild in 51.8% of the patients, moderate in 46.4%, and severe in 1.8%. Concerning duration, intermittent and persistent AC was observed in 51.6% and 48.4% of patients, respectively. The duration and severity of AC were similar in pediatric and adult populations (p = 0.947). Thus, AC was intermittent in 50.0% and 51.9% of the pediatric and adult populations, respectively; persistent in 50.0% and 48.0%; mild in 53.9% and 51.6%; moderate in 61.5% and 66.0%; and severe in 1.9% and 1.5% of the pediatric and adult populations, respectively (**table II**).

Figure 2 - *Association between AC and rhinitis or asthma.* ***(A)*** *Duration of AC and rhinitis (kappa = 0.544; p < 0.001 for both age groups);* ***(B)*** *Severity of AC and rhinitis (kappa = 0.363; p < 0.001 for both age groups);* ***(C)*** *Duration of AC and asthma in adults (kappa = 0.210; p < 0.001);* ***(D)*** *Severity of AC and asthma in adults (kappa = 0.281; p < 0.001).*



Comorbidities

The most frequent comorbidity was rhinitis in 88.4% of the patients (92.8% and 81.8% in the pediatric and adult groups, respectively, without a significant difference between the two age groups), followed by asthma in 38.2% of the patients (47.3% and 38.8% in the pediatric and adult groups, respectively, also without a significant difference between the two age groups) (**table II**). The third most frequent comorbidity was atopic dermatitis among patients in the pediatric group (9.5%, p < 0.001) and food allergy among adult patients (8.6%). Isolated AC was found only in 36 patients (4%), with no differences between children (3.0%) or adults (4.1%) (p = 0.66). When the development of allergic disease and comorbid pathologies was considered, food allergy was the first to appear in time, with a mean ± standard deviation time from onset of the symptoms to the time of the study of 19.0 ± 13.4 years, followed of atopic dermatitis (7.7 ± 10.8 years), rhinitis (5.0 ± 7.9 years), conjunctivitis (4.5 ± 7.2 years), and asthma (1.9 ± 2.1 years).

The association between AC with rhinitis or asthma in the adult and pediatric population is shown in **figure 2**. A moderate concordance was found between the duration of the conjunctivitis and allergic rhinitis (kappa = 0.544, p < 0.001) and a fair concordance (kappa = 0.363, p < 0.001) between the severity of both pathologies in the population (**figure 2 A, B**). We found that AC in adults has a fair concordance with asthma in severity (kappa = 0.281, p < 0.001) and duration (kappa = 0.210, p < 0.001) (**figure 2 C, D**). This association was not observed in the pediatric population either in duration (p = 0.111) or severity (p = 0.075).

Discussion

This study retrospectively explored the usefulness of the newly validated DECA classification of AC in both children and adults seeking consultations with allergy specialists in Spain. The study showed that, according to the DECA criteria, the most common presentations of AC were intermittent mild and

persistent moderate, both in pediatric and adult populations. It also revealed that allergic rhinitis and asthma were prevalent comorbidities of AC in all patients.

Concerning the prevalence of AC, this study showed that AC is highly prevalent (33%) among allergic patients in Spain. Similar rates of AC have been reported worldwide (3). In a prospective study of 458 allergic patients aged 5-15, 30% were diagnosed with AC (19), and there was also male predominance (63%), in agreement with our study. Despite the high prevalence of AC, it has been frequently ignored by both physicians and patients, which has resulted in underdiagnosed and undertreated patients, especially when it is associated with other allergic diseases such as rhinitis and/or asthma (20).

Overall, AC was mainly associated with rhinitis and asthma (88.3% and 38.3% of patients, respectively). The association was more prominent in children that in adults (93% with rhinitis and 47% with asthma). Similar results have been observed in related studies (19, 21). Also, studies have shown that AC presents isolated in only 5-6% of patients (22, 23), which is in agreement with our results (4% isolated AC). Likewise, other authors have reported that rhinitis without conjunctivitis is very infrequent (6.7%) (24).

It is well known that a family history of atopic diseases such as allergic rhinitis or allergic asthma increases the likelihood of other allergic disorders (25). The presence of ocular symptoms increases the role of rhinitis as a risk factor for asthma compared to rhinitis alone (26), but it is unknown whether the duration and severity of conjunctivitis could influence the severity and duration of its comorbidities. In this study, using the new AC classification, we have verified that, in adults, the greater the severity and duration of conjunctivitis, the greater the severity and duration of rhinitis and asthma. In the pediatric group, we also observed a significant correlation between the severity and duration of AC and rhinitis, which was found not significant in asthma.

We have not found any published study on the onset of AC in relation to its comorbidities, to place it chronologically in the so-called allergic march. Some studies have shown how rhinitis from the clinical point of view precedes asthma (27). In our study we indirectly found that rhinitis discreetly preceded conjunctivitis while the onset of asthma was later.

Following on the evidence that justifies allergic rhinitis and asthma as members of the "one airway, one disease" hypothesis, we suggest that there are epidemiological relationships, and severity and duration correlations between AC, rhinitis and asthma, which would allow the inclusion of AC in the "*united airway disease" concept*. The application of the new DECA classification for AC is consistent and complementary with that currently in use for rhinitis severity and duration, and could reduce the heterogeneity of the information on AC. The main limitations of this study are that it was not specifically designed for patients with AC and the retrospective nature of the analyses. It would be interesting to develop a prospective survey to carry out a detailed epidemiological study to better understand AC in the general population.

Conclusions

The DECA classification for AC has allowed direct relationships between AC, rhinitis, and asthma in terms of clinical severity and duration. This relationship can be considered as one more argument to include AC an integral part of the one airway concept.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

The authors acknowledge Francisco López de Saro, PhD (Trialance SCCL) for medical writing support.

References

1. Bielory L. Increasing the knowledge base of ocular allergy epidemiology. J Pediatr (Rio J). 2013;89(4):330-1. doi: 10.1016/j.jped.2013.05.001.
2. Shokouhi Shoormasti R, Pourpak Z, Fazlollahi MR, Kazemnejad A, Nadali F, Ebadi Z, *et al*. The Prevalence of Allergic Rhinitis, Allergic Conjunctivitis, Atopic Dermatitis and Asthma among Adults of Tehran. Iran J Public Health. 2018;47(11):1749-55. Available at: https://pubmed.ncbi.nlm.nih.gov/30581793/.
3. Miyazaki D, Fukagawa K, Okamoto S, Fukushima A, Uchio E, Ebihara N, *et al*. Epidemiological aspects of allergic conjunctivitis. Allergol Int. 2020;69(4):487-95. doi: 10.1016/j.alit.2020.06.004.
4. Bielory L. Allergic conjunctivitis and the impact of allergic rhinitis. Curr Allergy Asthma Rep. 2010;10(2):122-34. doi: 10.1007/s11882-010-0087-1.
5. Passalacqua G, Ciprandi G, Canonica GW. The nose-lung interaction in allergic rhinitis and asthma: united airways disease. Curr Opin Allergy Clin Immunol. 2001;1(1):7-13. doi: 10.1097/01.all.0000010978.62527.4e.
6. Hom MM, Bielory L. The anatomical and functional relationship between allergic conjunctivitis and allergic rhinitis. Allergy Rhinol (Providence). 2013;4(3):e110-9. doi: 10.2500/ar.2013.4.0067.
7. Khan DA. Allergic rhinitis and asthma: epidemiology and common pathophysiology. Allergy Asthma Proc. 2014;35(5):357-61. doi: 10.2500/aap.2014.35.3794.
8. Navarro AM, Delgado J, Muñoz-Cano RM, Dordal MT, Valero A, Quirce S, *et al*. Allergic respiratory disease (ARD), setting forth the basics: proposals of an expert consensus report. Clin Transl Allergy. 2017;7:16. doi: 10.1186/s13601-017-0150-2.
9. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008;63 Suppl 86:8-160. doi: 10.1111/j.1398-9995.2007.01620.x.
10. Deliu M, Belgrave D, Simpson A, Murray CS, Kerry G, Custovic A. Impact of rhinitis on asthma severity in school-age children. Allergy. 2014;69(11):1515-21. doi: 10.1111/all.12467.

11. Sánchez-Hernández MC, Montero J, Rondon C, Benitez del Castillo JM, Velázquez E, Herreras JM, *et al*. Consensus document on allergic conjunctivitis (DECA). J Investig Allergol Clin Immunol. 2015;25(2):94-106. Available at: https://pubmed.ncbi.nlm.nih.gov/25997302/.
12. Sánchez-Hernández MC, Navarro AM, Colás C, Del Cuvillo A, Sastre J, Mullol J, *et al*. Validation of the DECA criteria for allergic conjunctivitis severity and control. Clin Transl Allergy. 2020;10:43. doi: 10.1186/s13601-020-00349-4.
13. Sociedad Española de Alergología e Inmunología Clínica. Alergológica 2015: Factores epidemiológicos, clínicos y socioeconómicos de las enfermedades alérgicas en España. Draft Grupo de Comunicación Healthcare 2017.
14. Ojeda P, Sastre J, Olaguibel JM, Chivato T; investigators participating in the National Survey of the Spanish Society of Allergology and Clinical Immunology Alergológica 2015. Alergológica 2015: A National Survey on Allergic Diseases in the Adult Spanish Population. J Investig Allergol Clin Immunol. 2018;28(3):151-64. doi: 10.18176/jiaci.0264.
15. Ojeda P, Ibáñez MD, Olaguibel JM, Sastre J, Chivato T; investigators participating in the National Survey of the Spanish Society of Allergology and Clinical Immunology Alergológica 2015. Alergológica 2015: A National Survey on Allergic Diseases in the Spanish Pediatric Population. J Investig Allergol Clin Immunol. 2018;28(5):321-9. doi: 10.18176/jiaci.0308.
16. Valero A, Ferrer M, Sastre J, Navarro AM, Monclús L, Martí-Guadaño E, *et al*. A new criterion by which to discriminate between patients with moderate allergic rhinitis and patients with severe allergic rhinitis based on the Allergic Rhinitis and its Impact on Asthma severity items. J Allergy Clin Immunol. 2007;120(2):359-65. doi: 10.1016/j.jaci.2007.04.006.
17. Grupo Español para el Manejo del Asma. Guía Española para el Manejo del Asma (GEMA). Barcelona: Mayo 2003.
18. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74. Available at: https://pubmed.ncbi.nlm.nih.gov/843571/.
19. Gradman J, Wolthers OD. Allergic conjunctivitis in children with asthma, rhinitis and eczema in a secondary outpatient clinic. Pediatr Allergy Immunol. 2006;17(7):524-6. doi: 10.1111/j.1399-3038.2006.00429.x.
20. Neto HJ, Rosário NA, Westphal GL, Riedi CA, Santos HL. Allergic conjunctivitis in asthmatic children: as common as underreported. Ann Allergy Asthma Immunol. 2010 Nov;105(5):399-400. doi: 10.1016/j.anai.2010.08.020.
21. Palmares J, Delgado L, Cidade M, Quadrado MJ, Filipe HP; Season Study Group. Allergic conjunctivitis: a national cross-sectional study of clinical characteristics and quality of life. Eur J Ophthalmol. 2010;20(2):257-64. doi: 10.1177/112067211002000201.
22. Ibáñez MD, Garde JM. Allergy in patients under fourteen years of age in Alergológica 2005. J Investig Allergol Clin Immunol. 2009;19 Suppl 2:61-8. Available at: https://pubmed.ncbi.nlm.nih.gov/19530421/.
23. Singh K, Axelrod S, Bielory L. The epidemiology of ocular and nasal allergy in the United States, 1988-1994. J Allergy Clin Immunol. 2010;126(4):778-783.e6. doi: 10.1016/j.jaci.2010.06.050.
24. Wüthrich B, Brignoli R, Canevascini M, Gerber M. Epidemiological survey in hay fever patients: symptom prevalence and severity and influence on patient management. Schweiz Med Wochenschr. 1998;128(5):139-43. Available at: https://pubmed.ncbi.nlm.nih.gov/9522418/.
25. Dupuis P, Prokopich CL, Hynes A, Kim H. A contemporary look at allergic conjunctivitis. Allergy Asthma Clin Immunol. 2020;16:5. doi: 10.1186/s13223-020-0403-9.
26. Cibella F, Ferrante G, Cuttitta G, Bucchieri S, Melis MR, La Grutta S, *et al*. The burden of rhinitis and rhinoconjunctivitis in adolescents. Allergy Asthma Immunol Res. 2015;7(1):44-50. doi: 10.4168/aair.2015.7.1.44.
27. Greisner WA 3rd, Settipane RJ, Settipane GA. Co-existence of asthma and allergic rhinitis: a 23-year follow-up study of college students. Allergy Asthma Proc. 1998;19(4):185-8. doi: 10.2500/108854198778557836.

Eur Ann Allergy Clin Immunol Vol 54, N.6, 284-289, 2022

Z. Kanannejad[1], S. Alyasin[1,2], H. Esmaeilzadeh[1,2], H. Nabavizadeh[1,2], R. Amin[1,2]

Asthma and COVID-19 pandemic: focus on the eosinophil count and ACE2 expression

[1]Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Allergy and Clinical Immunology, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

Key words
Asthma; SARS-CoV-2; COVID-19; ACE2; eosinophil.

Corresponding authors
1) Soheila Alyasin
Allergy Research Center
Shiraz University of Medical Sciences
Mohammad Rasool Allah Research Tower
Khalili Street, Mulla Sadra Boulevard
Shiraz, Iran
ORCID ID: 0000-0001-7183-545X
E-mail: alyasins@sums.ac.ir
2) Hossein Esmaeilzadeh
Imam Hussain Square
Zand Street
Shiraz, Iran
ORCID ID: 0000-0003-0288-8181
E-mail: esmailzadeh_ho@yahoo.com

Doi
10.23822/EurAnnACI.1764-1489.233

Summary
Currently, the world is engaged with a coronavirus disease 2019 (COVID-19) caused by acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The Center for Disease Control and Prevention (CDC) has proposed moderate to severe asthma as a risk factor for COVID-19 susceptibility and severity. However, current evidences have not identified asthma in the top 10 comorbidities associated with COVID-19 fatalities. It raises the question that why patients with different type of asthma are not more vulnerable to SARS-CoV-2 infection like other respiratory infection. Increased number of eosinophils and elevated angiotensin-converting enzyme 2 (ACE2) expressions in asthma are supposed as two mechanisms which associated with decreased COVID-19 susceptibility in asthmatics. Some studies have been performed to evaluate two mentioned factors in asthmatic patients compared with healthy individuals. Herein, we address these mechanisms and investigate whether ACE2 and eosinophil could protect asthmatic patients against SARS-CoV-2 infection.

Impact statement
Increased ACE-2 expression and number of eosinophil might be an important predictor for reduced COVID-19 morbidity and mortality in asthmatic.

Introduction

SARS-CoV-2 is a causative agent of a new respiratory infection in human known as COVID-19 which is associated with pneumonia, cold, sneezing, and coughing. COVID-19 was first diagnosed and isolated from pneumonia patient who belongs to Wuhan, China, and then spread to other parts of China and other countries worldwide. On account of rapid spread, WHO reported it as a Public Health Emergency of International Concern (PHEIC) on 30 January 2020 (1). At the time of writing, the daily incidence of COVID-19 was 7,690,708 confirmed cases, with 427,630 deaths worldwide. Therefore, the identification of risks and protective factors for disease severity from COVID-19 is critical to the direct development of new treatments and infection prevention strategies. Evidence has identified that patients with diabetes, hypertension, obesity, chronic kidney disease, liver disease, immunocompromised disease, hemoglobin disorder, and cardiovascular diseases are more susceptible to COVID-19 infection and worse survival than other populations (2). In addition to such conditions, patients with asthma and allergic respiratory diseases have been proposed as higher risk populations for COVID-19 infection by CDC (3). It is based on the fact that other respiratory viral infections created by influenza and rhinovirus could affect allergic patients and those with chronic respiratory diseases such as asthma more than healthy individuals (4). Studies have shown that patients with asthma have impaired innate immune responses and interferon gamma (IFN-γ) production against respiratory virus-

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

es compared with non-asthmatics (5). Therefore, these days there are some concerns and issues about allergic patients due to COVID-19 pandemic. However, existing studies have not indicated a high prevalence of asthma among patients with COVID-19 infection (6, 7). It is hypothesized that asthmatic individuals are relatively resistant to COVID-19 infection due to the features of the disease or conventional asthma treatment. The purpose of this work is to discuss how COVID-19 pandemic affect asthmatic and what could be the explanation for the paucity of asthmatic in patients with COVID-19.

Evidence acquisition

A literature search in PubMed databases was separately conducted by two researchers using the following "COVID-19" OR "SARS-CoV-2" OR "Coronavirus" AND "Asthma" OR "Allergy" AND "Case series" OR "Comorbidities" OR "Epidemiology" OR "Hospitalization" OR "Risk factors." to assess how asthma is affected by COVID-19 pandemic and "Eosinophil" and "ACE2" in the English language up to November 2020 and then updated in May 2021. Relevant studies with these keywords were included in this study.

Results

Prevalence of asthma among patients with COVID-19 infection

Some clinical-epidemiological studies have performed to evaluate the risk of serious adverse outcomes in patients with COVID-19 by stratification according to the number and type of comorbidities and identified some sub-populations with poorer prognosis (8-10). A meta-analysis study on 43 studies involving 3600 patients in China has been shown that 12.0% to 67.0% laboratory-confirmed COVID-19 cases have coexisting medical comorbidity (2). In this study the most prevalent comorbidities have noted as hypertension ranged from 0.0% to 48.0% (median 16.0%; 27 studies), diabetes ranged from 0.0% to 50.0% (median 10.1%; 26 studies), cancer ranged from 0.0% to 17.0% (median 1.0%; 15 studies), chronic respiratory/lung diseases ranged from 0.0% to 17.0% (median 2.0%; 16 studies) (2). It was reasonable to anticipate that patients with chronic respiratory diseases would be more susceptible to a more severe viral infection and development of acute respiratory distress syndrome (ARDS) that can complicate SARS-CoV-2 pneumonia. On the contrary, among all the series of COVID-19 (mild or severe presentation alike), except one study conducted in the United States, the prevalence of patients with chronic asthma or with common related diseases such as allergic rhinitis or atopy was low and failed to exceed general population (11). In a US-based study, the prevalence of chronic lung disease (primarily asthma) was 34.6% among 178 hospitalized adult patients with COVID-19 (6). This unexpectedly high reported numbers of chronic lung disease prevalence among patients with COVID-19 may be owing to the small proportion of the hospitalized patients with COVID-19 (approximately 12%) included in this study While, in another study performed in the US, asthma was documented in 12.5% of 393 hospitalized patients with SARS-CoV-2 infection and chronic obstructive pulmonary disease (COPD) in 5.1% (7).

Some studies have tried to compare the clinical outcomes of COVID-19 infection in patients with or without asthma (12, 13). Wang *et al*. performed a meta-analysis based on 4 studies from 744 patients with asthma and 8,151 patients without asthma and reported that asthma had no significant effect on mortality (14). Due to limited data in previous study, Wang *et al*. performed a meta-analysis study based on 14 publications with 17,694 precipitants and found that severe COVID-19 was not associated with an increased risk of asthma compared with non-severe COVID-19 while asthma was not associated with increased risk of mortality in patients with COVID-19 (15). In this context, a recent systematic and meta-analysis review performed by Liu *et al*. has compared the COVID-19 outcomes between patients with and without asthma based on 131 studies. They showed that asthma is not associated with higher COVID-19 severity or worse prognosis, and patients with asthma are found to have a lower risk of death compared with patients without asthma (16). It raises the question of why SARS-CoV-2 is associated with potential for reduced severity of COVID-19 infection in patients with asthma unlike other respiratory viruses such as influenza and rhinovirus that affect both adult (7.6%-46%) and children (8.3%-42%) with asthma (17-19). Recently, some asthma features including reduced angiotensin-converting enzyme 2 (ACE2) gene expressions in airway cells, and eosinophilia have been proposed as protective mechanisms against COVID-19 susceptibility and severity. For future literature review, we aimed to assay protective effects of these mechanisms in the context of COVID-19.

Asthma, COVID-19, and ACE2 expression

ACE2 is an enzyme attached to the cell membranes of cells in the lungs, arteries, heart, kidney, and intestines which processes angiotensin (Ang) I and II into Ang (1-10) and (1-8), respectively. Ang (1-8) peptide has been shown to mediate vasodilatative (hypotension), antiproliferative and apoptotic effects through Mas receptor (20). ACE2 serves as a cellular receptor for some coronaviruses, including HCoV-NL63, SARS-CoV and SARS-CoV-2 (21). More specifically, the binding of the spike S1 protein of viruses to the enzymatic domain of ACE2 on the surface of cells results in endocytosis and translocation of both the virus and the enzyme into endosomes located within cells. This entry process also requires priming of the S protein by the host serine protease TMPRSS2, the inhibition of which is under current investigation as a potential therapeutic (22).

Some studies have tried to investigate the relationship between ACE2 expression as the main host cell receptor and COVID-19 susceptibility in different populations. It has been shown that higher ACE2 expression in some situations such as smoking, diabetes, and hypertension increases *in vitro* susceptibility to SARS-CoV-2 infection, while its down-regulation in children is associated with decreased susceptibility (23, 24). Given the lower risk of COVID-19 among asthmatics, it is hypothesized that it may be due to differential expression of ACE2 in asthma. Therefore, some studies have performed to analyze ACE2 levels in asthmatics compared with non-asthmatics. First study has performed by Jackson *et al.*, it has been shown that respiratory allergy and controlled allergen exposures are associated with significant reductions in ACE2 expression (25). Another study by Song *et al.* has also reported decreased ACE2 gene and protein expression in asthmatics compared to healthy control (26). Evidence has shown that Type-2 inflammation modulates ACE2 expression in airway epithelial cells in asthma. A study reported that IL-13 exposure reduced ACE2 and increased transmembrane serine protease 2 expressions in airway epithelial cells from patients with asthma and atopy (27). In sharp contrast, *in vitro* treatment of airway epithelial cells with IFNs enhanced their ACE2 expression (28). Contrary to previous studies, there are some evidences that show no significant difference for ACE2 mRNA expression between asthmatic patients and healthy controls (29, 30). However, these conflicting results may be due the known heterogeneity in asthma. In a recent study, Camiolo *et al.* has reported that expression of ACE2 among asthmatic patients varies by asthma inflammatory endotypes. They showed that ACE2 gene expression in a subset of type-2 low asthmatics (low blood eosinophil and increased type-1 immunity) is higher than type-2 High asthma (high blood eosinophil and increased type 2 immunity) (31). In addition to type-2 low asthma endotype, increased expression of ACE-2 has been reported in type2-low phenotypes including obesity, smoking, and age associated asthma (32). Using asthma treatments should be also considered as main factor related to ACE-2 level. Low dose, inhaled corticosteroids (ICS) might exert protective effects on asthma patients by reducing airway inflammation, ACE2 and TMPRSS2 expression in SARS-CoV infection (33). However, some studies reported increased airway ACE2 expression in asthmatics on long-term treatment with ICS (27, 34). Other confounding factors such as male sex, African-American ethnicity, and a history of diabetes mellitus or other comorbidities may affect ACE2 expression among asthmatics (33).

In contrast with SARS-CoV-2, conventional coronaviruses and some respiratory viruses especially rhinovirus, which exacerbate asthma upon infection use different entry receptors from ACE2. The reported receptors for conventional coronaviruses are HLA class I molecule, and caveolin-1 for HCoV-OC43; aminopeptidase N (CD13) for HCoV-229E; dipeptidyl peptidase 4 (CD26) for HCoV-EMC; and intercellular adhesion molecule 1 (ICAM-1) for rhinovirus, which expression is high in allergic airways as a marker of allergic inflammation (35). Thus, varied effects on respiratory allergic diseases between SARS-CoV-2 and other respiratory viruses may be due to using different molecular receptors expressed by respiratory epithelial cells.

Asthma, COVID-19, and eosinophils

Eosinophils are special polymorphonuclear leukocytes. They develop in the bone marrow and migrate into blood, making up about 1-6% of white blood cells. The presence of large specific granules including major basic protein, eosinophil peroxidase, and 2 RNAses (eosinophil cationic protein and eosinophil neurotoxin) are characteristic features of these cells. In addition to their pro-inflammatory effects, pieces of evidence have revealed that eosinophils play pleiotropic roles as regulatory cells involved in protective immunity, including antiviral responses and shaping diverse physiological responses, such as organ development and metabolism (36). It has been documented by preclinical studies (mainly in mice) that eosinophils have the main role against respiratory viruses via endosomal Toll-like receptors (TLRs) - including TLR3, TLR7, and TLR9 - eosinophil-derived neurotoxin (EDN/RNAse2) and eosinophil cationic protein (ECP/RNAse3), nitric oxide (NO) production via inducible NO synthase, MHC-I and CD86 up-regulation, and cytokines production, which enable them to recognize, respond and orchestrate effective responses (37). Studies showed that the adoptive transfer of eosinophils from *Aspergillus fumigatus* antigen-sensitized mice into the airways of influenza virus-infected mice decreases viral titers and increases virus-specific $CD8^+$ T cells in comparison to that of animals who did not receive eosinophils (38). Also, human subjects with asthma were treated with the anti-eosinophil drug mepolizumab (an anti–IL-5 humanized mAb) or placebo, and subsequently challenged with rhinovirus; mepolizumab-treated patients demonstrated significant increases in their rhinovirus viral titers in the upper airway, supporting an antiviral role for eosinophils (39).

There is little indication that eosinophils have a protective or exacerbating role during SARS-CoV-2 infection. However, based on some evidences, it seems that eosinophils may play a protective role, and decreased eosinophils levels (eosinopenia) are associated with a poor prognosis in patients with COVID-19 (40). Liu *et al.* reported that more than half the patients admitted with COVID-19 (53%) had eosinopenia (defined as absolute eosinophil counts $< 0.02 \times 10^9$ cells/L) on the day of hospital admission (40). Similarly, medical records of 85 fatal cases of COVID-19 showed that 81% of the patients had absolute eosinophil counts below the normal range (absolute eosinophil counts $< 0.02 \times 10^9$ cells/L) at the time of admission (41). Notably, eosinophils levels improved in all patients

before discharge, suggesting that resolution of eosinopenia may be an indicator of improving clinical status in patients with COVID-19 (40). The exact immune mechanism performed by eosinophils against coronaviruses has remained unknown and most studies are focused on other respiratory viruses such as influenza, rhinovirus, and human orthopneumovirus (RSV) (37). However, some literatures have pointed to TLR7, EDN, ECP, increased MHC-I, and CD86 expression as the potential immune mechanisms used by eosinophils against single strand RNA (ssRNA) viruses like SARS-CoV-2 (42). TLR-7 recognizes ssRNA and its expression is higher in eosinophils compared with neutrophils, suggesting the possible antiviral activity of this cell against ssRNA viruses. TLR-7 stimulation is associated with eosinophil cytokine production, degranulation, superoxide, and NO generation (37). In addition, ECP along with EDN reduce the infectivity of SARS-CoV-2 by a ribonuclease-dependent mechanism (43). Moreover, increased MHC-I and CD86 expression by eosinophils enable them to directly interact with $CD8^+$ T cells and promote the recruitment of virus-specific $CD8^+$ T cells into the lungs to enhance antiviral immunity (38). As approximately 50-70% of asthmatic patients have Th2 high/eosinophilic asthma, eosinophilia may be a reason for reducing COVID-19 susceptibility among these patients. There are limited studies investigated role of eosinophilia in COVID-19 susceptibility and outcomes for asthmatic population. Ferastraoaru *et al.* showed that in asthmatics, pre-existing eosinophilia (AEC ≥ 150 cells/mL) was protective from severe COVID-19 infection, and also development of eosinophilia (AEC ≥ 150 cells/mL) during hospitalization was associated with decreased mortality (44). However, some studies reported that eosinophilia, both in those with and without asthma, may be associated with reduced mortality risk (45, 46).

Discussion

Regarding the pathophysiology of asthma, it seems reasonable to consider asthma as a risk factor for higher susceptibility and severity of COVID-19 infection. Patients with asthma have deficient viral immune responses due to impaired interferon production that predispose them to increase susceptibility to viral infection (47). These patients have also a tendency for severe form of viral respiratory tract infections associated with adverse outcomes (48). Despite the concern that patients with asthma might suffer from severe form of COVID-19 infection, the results of most clinical-epidemiological studies did not indicate asthma as a risk factor for COVID-19 (16, 17). It hypothesized that asthma features including reduced ACE-2 expression, type 2 immune response, eosinophilia, and conventional therapeutics might provide potential protective effects against infection with SARS-CoV-2. In the current study, we reviewed studies that investigated ACE-2 expression and eosinophil in asthma related to COVID-19 infection.

Most studies have reported decreased ACE-2 expression in patients with allergic asthma. However, it should be considered that ACE-2 expression in asthmatic is varied and might be influenced by asthma endotypes (type2-high or type2-low), phenotypes (non-atopic and atopic), treatment, ethnicity and other comorbidities. These mentioned factors may be a reason for reporting different proportion of asthma patients in US and China (7). Therefore, these factors should be considered in studies that investigated ACE2 level and COVID-19 susceptibility among asthmatics.

Eosinophils in the respiratory tract might represent a "double-edged sword" response against some respiratory viruses. Eosinophil promotes antiviral responses against some respiratory viruses through the release of RNAses and reactive nitrogen species, while it dysregulated responses during allergic disease given their increased numbers and/or activation status, ultimately resulting in an exaggerated host response that can lead to host tissue damage (36). In the context of COVID-19, it seems that eosinophil may play a protective role, and eosinopenia has been noted to be a marker of early severe COVID-19 disease, which may result from eosinophils exhaustion, viral inhibition of eosinophils production, or induction of eosinophils apoptosis (44). Like to ACE-2 expression, different asthma endotypes might modify eosinophil counts differently, thereby affecting COVID-19 outcomes. Eosinophilia has reported in type-2 high asthma, while type-2 low asthma has generally characterized with neutrophilic inflammation (49). There is no study that investigated COVID-19 outcomes in varied asthma endotypes regarding eosinophil counts or their metabolites. Future studies are needed to help better distinguishing the impact of different asthma phenotypes and comorbidities on COVID-19 outcomes. In the current work, we have tried to include all important relevant papers (but not necessarily every paper written on the topic). Therefore, some relevant article might be left out due to space limitations.

Conclusions

In summary, it seems that having a Th2-asthma phenotype associated with increases ACE-2 expression and eosinophilia might be an important predictor for reduced COVID-19 morbidity and mortality in asthmatic that need to be more investigated in prospective and mechanistic studies.

Fundings

This study was financially supported by Grant (No 99-01-106-22305) from Shiraz University of Medical Sciences.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433. doi: 10.1016/j.jaut.2020.102433.
2. Fu L, Wang B, Yuan T, Chen X, Ao Y, Fitzpatrick T, *et al*. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: a systematic review and meta-analysis. J Infect. 2020;80(6):656-65. doi: 10.1016/j.jinf.2020.03.041.
3. Centers for Disease Control and Prevention. People with certain medical conditions. 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html. Last access date: 01/31/2021.
4. Jartti T, Bønnelykke K, Elenius V, Feleszko W (Eds). Role of viruses in asthma. Seminars in immunopathology. Springer, 2020.
5. Bergauer A, Sopel N, Kroß B, Vuorinen T, Xepapadaki P, Weiss ST, *et al*. IFN-α/IFN-λ responses to respiratory viruses in paediatric asthma. Eur Respir J. 2017;49(2):1600969. doi: 10.1183/13993003.00969-2016.
6. Garg S, Kim L, Whitaker M, O'Halloran A, Cummings C, Holstein R, *et al*. Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 - COVID-NET, 14 States, March 1-30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(15):458-64. doi: 10.15585/mmwr.mm6915e3.
7. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, *et al*. Clinical Characteristics of Covid-19 in New York City. N Engl J Med. 2020;382(24):2372-4. doi: 10.1056/NEJMc2010419.
8. Guan WJ, Liang WH, He JX, Zhong NS. Cardiovascular comorbidity and its impact on patients with COVID-19. Eur Respir J. 2020;55(6):2001227. doi: 10.1183/13993003.01227-2020.
9. Jin X, Lian J-S, Hu J-H, Gao J, Zheng L, Zhang Y-M, *et al*. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. Gut. 2020;69(6):1002-9. doi: 10.1136/gutjnl-2020-320926.
10. Liang W-h, Guan W-j, Li C-c, Li Y-m, Liang H-r, Zhao Y, *et al*. Clinical characteristics and outcomes of hospitalised patients with COVID-19 treated in Hubei (epicentre) and outside Hubei (non-epicentre): a nationwide analysis of China. Eur Respir J. 2020;55(6):2000562. doi: 10.1183/13993003.00562-2020.
11. Naziroğlu T, Aksu K. Rare atopy in COVID-19 patients or COVID-19 famine in atopic patients? Dermatol Ther. 2021;34(1):e14581. doi: 10.1111/dth.14581.
12. Mahdavinia M, Foster KJ, Jauregui E, Moore D, Adnan D, Andy-Nweye AB, Khan S, Bishehsari F. Asthma prolongs intubation in COVID-19. J Allergy Clin Immunol Pract. 2020;8(7):2388-91. doi: 10.1016/j.jaip.2020.05.006.
13. Chhiba KD, Patel GB, Vu THT, Chen MM, Guo A, Kudlaty E, *et al*. Prevalence and characterization of asthma in hospitalized and nonhospitalized patients with COVID-19. J Allergy Clin Immunol. 2020;146(2):307-14.e4. doi: 10.1016/j.jaci.2020.06.010.
14. Wang Y, Chen J, Chen W, Liu L, Dong M, Ji J, *et al*. Does Asthma Increase the Mortality of Patients with COVID-19?: A Systematic Review and Meta-Analysis. Int Arch Allergy Immunol. 2021;182(1):76-82. doi: 10.1159/000510953.
15. Wang Y, Ao G, Qi X, Xie B. The association between COVID-19 and asthma: A systematic review and meta-analysis. Clin Exp Allergy. 2020;50(11):1274-7. doi: 10.1111/cea.13733.
16. Liu S, Cao Y, Du T, Zhi Y. Prevalence of Comorbid Asthma and Related Outcomes in COVID-19: A Systematic Review and Meta-Analysis. J Allergy Clin Immunol Pract. 2021;9(2):693-701. doi: 10.1016/j.jaip.2020.11.054.
17. Akdis C, PW PWH, Agache I. Global atlas of allergic rhinitis and chronic rhinosinusitis. EAACI. 2015. Available at: https://hub.eaaci.org/education_books/global-atlas-of-allergic-rhinitis-and-chronic-rhinosinusitis/.
18. Centers for Disease Control and Prevention (CDC). Patients hospitalized with 2009 pandemic influenza A (H1N1) - New York City, May 2009. MMWR Morb Mortal Wkly Rep. 2010;58(51):1436-40. Available at: https://pubmed.ncbi.nlm.nih.gov/20057350/.
19. Dawood FS, Fiore A, Kamimoto L, Bramley A, Reingold A, Gershman K, *et al*. Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008. J Pediatr. 2010;157(5):808-14. doi: 10.1016/j.jpeds.2010.05.012.
20. Jia H. Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease. Shock. 2016;46(3):239-48. doi: 10.1097/SHK.0000000000000633.
21. Aleksova A, Ferro F, Gagno G, Cappelletto C, Santon D, Rossi M, *et al*. COVID-19 and renin-angiotensin system inhibition: role of angiotensin converting enzyme 2 (ACE2)-Is there any scientific evidence for controversy? J Intern Med. 2020;288(4):410-21. doi: 10.1111/joim.13101.
22. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, *et al*. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052.
23. Ghafouri-Fard S, Noroozi R, Omrani MD, Branicki W, Pośpiech E, Sayad A, *et al*. Angiotensin converting enzyme: A review on expression profile and its association with human disorders with special focus on SARS-CoV-2 infection. Vascul Pharmacol. 2020;130:106680. doi: 10.1016/j.vph.2020.106680.
24. Bunyavanich S, Do A, Vicencio A. Nasal Gene Expression of Angiotensin-Converting Enzyme 2 in Children and Adults. JAMA. 2020;323(23):2427-9. doi: 10.1001/jama.2020.8707.
25. Jackson DJ, Busse WW, Bacharier LB, Kattan M, O'Connor GT, Wood RA, *et al*. Association of respiratory allergy, asthma and expression of the SARS-CoV-2 receptor ACE2. J Allergy Clin Immunol. 2020;146(1):203-206.e3. doi: 10.1016/j.jaci.2020.04.009.
26. Song J, Zeng M, Wang H, Qin C, Hou HY, Sun ZY, *et al.* Distinct effects of asthma and COPD comorbidity on disease expression and outcome in patients with COVID-19. Allergy. 2021 Feb;76(2):483-496. doi: 10.1111/all.14517.
27. Kimura H, Francisco D, Conway M, Martinez FD, Vercelli D, Polverino F, *et al*. Type 2 inflammation modulates ACE2 and TMPRSS2 in airway epithelial cells. J Allergy Clin Immunol. 2020;146(1):80-8.e8. doi: 10.1016/j.jaci.2020.05.004.
28. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, *et al.* SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell. 2020;181(5):1016-1035.e19. doi: 10.1016/j.cell.2020.04.035.

29. Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, *et al*. Distribution of ACE2, CD147, CD26 and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. Allergy. 2020;75(11):2829-45. doi: 10.1111/all.14429.
30. Bradding P, Richardson M, Hinks TSC, Howarth PH, Choy DF, Arron JR, *et al*. ACE2, TMPRSS2, and furin gene expression in the airways of people with asthma-implications for COVID-19. J Allergy Clin Immunol. 2020;146(1):208-11. doi: 10.1016/j.jaci.2020.05.013.
31. Camiolo M, Gauthier M, Kaminski N, Ray A, Wenzel SE. Expression of SARS-CoV-2 receptor ACE2 and coincident host response signature varies by asthma inflammatory phenotype. J Allergy Clin Immunol. 2020;146(2):315-24.e7. doi: 10.1016/j.jaci.2020.05.051.
32. Cai G, Bossé Y, Xiao F, Kheradmand F, Amos CI. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. Am J Respir Crit Care Med. 2020;201(12):1557-9. doi: 10.1164/rccm.202003-0693LE.
33. Peters MC, Sajuthi S, Deford P, Christenson S, Rios CL, Montgomery MT, *et al*. COVID-19-related Genes in Sputum Cells in Asthma. Relationship to Demographic Features and Corticosteroids. Am J Respir Crit Care Med. 2020;202(1):83-90. doi: 10.1164/rccm.202003-0821OC.
34. O'Beirne SL, Salit J, Kaner RJ, Crystal RG, Strulovici-Barel Y. Up-regulation of ACE2, the SARS-CoV-2 receptor, in asthmatics on maintenance inhaled corticosteroids. Respir Res. 2021;22(1):200. doi: 10.1186/s12931-021-01782-0.
35. Zhou X, Zhu L, Lizarraga R, Chen Y. Human Airway Epithelial Cells Direct Significant Rhinovirus Replication in Monocytic Cells by Enhancing ICAM1 Expression. Am J Respir Cell Mol Biol. 2017;57(2):216-25. doi: 10.1165/rcmb.2016-0271OC.
36. McBrien CN, Menzies-Gow A. The Biology of Eosinophils and Their Role in Asthma. Front Med (Lausanne). 2017;4:93. doi: 10.3389/fmed.2017.00093.
37. Flores-Torres AS, Salinas-Carmona MC, Salinas E, Rosas-Taraco AG. Eosinophils and Respiratory Viruses. Viral Immunol. 2019;32(5):198-207. doi: 10.1089/vim.2018.0150.
38. Samarasinghe AE, Melo RC, Duan S, LeMessurier KS, Liedmann S, Surman SL, *et al*. Eosinophils Promote Antiviral Immunity in Mice Infected with Influenza A Virus. J Immunol. 2017;198(8):3214-26. doi: 10.4049/jimmunol.1600787.
39. Sabogal Piñeros YS, Bal SM, van de Pol MA, Dierdorp BS, Dekker T, Dijkhuis A, *et al*. Anti–IL-5 in Mild Asthma Alters Rhinovirus-induced Macrophage, B-Cell, and Neutrophil Responses (MATERIAL). A Placebo-controlled, Double-Blind Study. Am J Respir Crit Care Med. 2019;199(4):508-17. doi: 10.1164/rccm.201803-0461OC.
40. Liu F, Xu A, Zhang Y, Xuan W, Yan T, Pan K, Yu W, Zhang J. Patients of COVID-19 may benefit from sustained Lopinavir-combined regimen and the increase of Eosinophil may predict the outcome of COVID-19 progression. Int J Infect Dis. 2020;95:183-91. doi: 10.1016/j.ijid.2020.03.013.
41. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, *et al*. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study. Am J Respir Crit Care Med. 2020;201(11):1372-9. doi: 10.1164/rccm.202003-0543OC.
42. Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther. 2020;5(1):84. doi: 10.1038/s41392-020-0191-1.
43. Lindsley AW, Schwartz JT, Rothenberg ME. Eosinophil responses during COVID-19 infections and coronavirus vaccination. J Allergy Clin Immunol. 2020;146(1):1-7. doi: 10.1016/j.jaci.2020.04.021.
44. Ferastraoaru D, Hudes G, Jerschow E, Jariwala S, Karagic M, de Vos G, *et al*. Eosinophilia in Asthma Patients Is Protective Against Severe COVID-19 Illness. J Allergy Clin Immunol Pract. 2021;9(3):1152-62.e3. doi: 10.1016/j.jaip.2020.12.045.
45. Eggert LE, He Z, Collins W, Lee AS, Dhondalay G, Jiang SY, *et al*. Asthma phenotypes, associated comorbidities, and long-term symptoms in COVID-19. Allergy. 2022;77(1):173-85. doi: 10.1111/all.14972.
46. Ho KS, Howell D, Rogers L, Narasimhan B, Verma H, Steiger D. The relationship between asthma, eosinophilia, and outcomes in coronavirus disease 2019 infection. Ann Allergy Asthma Immunol. 2021;127(1):42-8. doi: 10.1016/j.anai.2021.02.021.
47. Gonzales-van Horn SR, Farrar JD. Interferon at the crossroads of allergy and viral infections. J Leukoc Biol. 2015;98(2):185-94. doi: 10.1189/jlb.3RU0315-099R.
48. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet. 2010;376(9743):826-34. doi: 10.1016/S0140-6736(10)61380-3.
49. Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clin Rev Allergy Immunol. 2019;56(2):219-33. doi: 10.1007/s12016-018-8712-1.

A. M. Mesquita, R. Moço Coutinho, L. Amaral, J. L. Plácido, A. Coimbra

Sensitization to bee venom in Portuguese non-allergic beekeepers

Department of Allergy and Clinical Immunology, University Hospital Center of São João (CHUSJ), Porto, Portugal

Key words

Beekeepers; bee venom; sensitization; non-allergic; sting.

Corresponding author
Ana Margarida Mesquita
Department of Allergy and Clinical Immunology
University Hospital Center of São João (CHUSJ)
Alameda Prof. Hernâni Monteiro
4200-319 Porto, Portugal
ORCID ID: 0000-0002-8790-6475
E-mail: mesquita.amb@gmail.com

Doi
10.23822/EurAnnACI.1764-1489.223

To the Editor,

the prevalence of hymenoptera stings in the general population ranges from 56.6% to 94.5%, and it may vary according to the location and the climatic conditions, whereas the estimated prevalence of hymenoptera venom allergy is approximatly 5% (1). Allergy to hymenoptera venom including honeybee (*Apis mellifera*) is one of the main causes of anaphylaxis both in adults and children (2). In professionals such as beekeepers, gardeners, farmers, truck drivers and masons, venom allergy is considered an occupational allergy and its occurrence exceeds that of the general population due to higher exposure to the respective insect (1, 3). Beekeepers and their family members are especially at risk of developing allergic sting reactions. Reported data suggests that 14-32% of beekeepers are allergic to bee venom and definite risk factors are the first years of beekeeping, fewer than 10 annual bee stings, high skin sensitivity and serum-specific IgE to bee venom and low serum venom-specific IgG as well as a history of atopy (4-6).

The objective of this study was to evaluate the sensitization to bee venom in beekeepers without any history of systemic reactions to bee stings.

Subjects were eligible for inclusion if they were beekeepers, at least 18 years old, able to consent and did not have a history of sting-induced systemic reactions. The participants were recruited during a beekeeping meeting in 2018. This article complies with the principles of the Declaration of Helsinki.

A structured questionnaire was used to collect demographic data and history of atopic diseases (atopic dermatitis, allergic rhinoconjunctivitis and/or allergic asthma) or any other medical condition, duration of beekeeping, the use of protective suits and information on previous hymenoptera stings (number and local of stings, time interval to last sting, history of large local or systemic sting reactions). Large local reactions were defined as greater than 10 cm in diameter and persistence for more than 24 hours (7). Subjects reporting systemic sting reactions were excluded from evaluation.

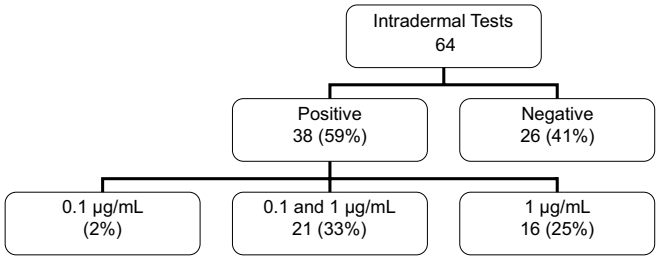
Skin prick tests were performed with aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cultivated and wild grasses, *Olea europaea* and *Parietaria judaica*) and intradermal tests with bee venom at two concentrations, 0.1 and 1 mg/mL.

Statistical analysis was performed with SPSS version 24 for Windows (SPSS Inc., Chicago, Ill). Interval-scaled data are presented as median and interquartile range (IQR). Ordinally and categorically scaled data are reported as absolute and relative frequencies and P-values < 0.05 were considered statistically significant.

A total of 64 beekeepers without any history of systemic reactions to bee stings agreed to participate. Fifty-two (81%) were male, with a median age of 46 (± 15) years. Nine (14%) reported rhinitis and 4 (6%) asthma. Duration of beekeeping activity

© 2022 Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri - AAIITO. Published by EDRA SpA. All rights reserved.

***Figure 1** - Intradermal tests in beekeepers without hymenoptera venom allergy.*



was as follows: 5 (8%) under 1 year, 10 (16%) 1 to 2 years, 18 (28%) 2 to 5 years, 13 (20%) 5 to 10 years and 18 (28%) longer than 10 years. With respect to the use of protective suits, 83% reported that they always wore them; 14% sometimes wore them and 3% admitted not using any protective gear.

Skin prick tests with common aeroallergens were positive to *D. pteronyssinus* in 9%, cultivated grass pollens 8%, wild grass pollens 9%; olive tree (*Olea europaea*) 5% and *Parietaria judaica* 2%.

Of the total, 38 (59%) had positive IDT with bee venom: 1 (2%) positive with 0.1 mg/mL, 16 (25%) with 1 mg/mL and 21 (33%) with both concentrations (**figure 1**).

In beekeepers with less than one year of activity, 3/5 (60%) had positive IDT, while 9/18 (50%) of those with over 10 years had positive IDT, so in this study there were no statistically significant differences between sensitivity and the number of years of beekeeping.

The beekeepers who affirmed wearing totally protective suits were less sensitized to bee venom ($p = 0.011$). Those with more years of beekeeping had a higher number of positive IDT with 0.1 mg/mL ($p < 0.05$). In addition, sensitization to cultivated grass pollen and wild grass pollen was associated with a higher number of positive IDT with 0.1 mg/mL ($p = 0.001$ and $p = 0.048$, respectively) and 1 mg/mL ($p = 0.007$ and $p = 0.028$, respectively). In this group, there was no significant association between the estimated mean annual number of stings and sensitization to bee venom.

In this sample, 59% of the beekeepers without any systemic reactions were sensitized to bee venom. This may be explained by the greater exposure to stings when no protective suit is worn and a longer period of beekeeping.

Regular exposure to bee venom in these individuals may confer greater tolerance and thus reduce the risk of systemic allergic reactions with stings. The follow-up of these beekeepers would allow to observe which ones will become allergic and if the previous sensitization to bee venom is a predictive or protective factor of allergy.

More extensive studies with larger samples and follow-up time may help to clarify these issues.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Toletone A, Voltolini S, Passalacqua G, Dini G, Bignardi D, Minale P, *et al.* Hymenoptera venom allergy in outdoor workers: Occupational exposure, clinical features and effects of allergen immunotherapy. Hum Vaccin Immunother. 2017;13(2):477-83. doi: 10.1080/21645515.2017.
2. Matysiak J, Matysiak J, Bręborowicz A, Kycler Z, Dereziński P, Kokot ZJ. Immune and clinical response to honeybee venom in beekeepers. Ann Agric Environ Med. 2016;23(1):120-4. doi: 10.5604/12321966.1196866.
3. Tomsitz D, Brockow K. Component Resolved Diagnosis in Hymenoptera Anaphylaxis. Curr Allergy Asthma Rep. 2017;17(6):38. doi: 10.1007/s11882-017-0707-0.
4. Müller UR. Bee venom allergy in beekeepers and their family members. Curr Opin Allergy Clin Immunol. 2005;5(4):343-7. doi: 10.1097/01.all.0000173783.42906.95.
5. Bousquet J, Ménardo JL, Aznar R, Robinet-Lévy M, Michel FB. Clinical and immunologic survey in beekeepers in relation to their sensitization. J Allergy Clin Immunol. 1984;73(3):332-40. doi: 10.1016/0091-6749(84)90405-6.
6. Eich-Wanger C, Muller UR. Bee sting allergy in beekeepers. Clin Exp Allergy. 1998;28(10):1292-8. doi: 10.1046/j.1365-2222.1998.00411.x
7. Bilo BM, Ruëff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy. 2005;60(11):1339-49. doi: 10.1111/j.1398-9995.2005.00963.x.

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: http://eaaci.edmgr.com.
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 in the print journal and 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: www.eurannallergyimm.com.
Typed manuscripts at 30 lines per page: maximum lenght 10 pages, around 300 lines.
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. Generally, the study (Text) must present the word line numbers and should be divided into the following parts and in the order indicated:

1. **Summary, key words and impact statement (the last one to avoid in the Letters to the Editor)**.
2. **Introduction**: containing the reasons for doing the work.
3. **Materials and methods**.
4. **Results**: these should be given concisely; the use of tables and figures to illustrate the same results will only rarely be allowed.
5. **Discussion**: the presentation of results should be separated from a discussion of their significance.
6. **References**.

Units and Abbreviations

European Annals of Allergy and Clinical Immunology recognizes the adoption of the International Systems of Units (SI-Units). Abbreviations have to be put in a glossary at the foot of page 1 of the text.

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.

For example:
Bodtger U, Linnegerg A. Remission of allergic rhinitis: An 8-year observational study. J Allergy Clin Immunol 2004; 114(6): 1384-1388.

- 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: 324-342.

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 SpA
Via Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edraspa.it

L'INFORMATORE FARMACEUTICO

2022

LA DIFFICOLTÀ DI ALCUNE SCELTE, LA CERTEZZA DI POTERSI AFFIDARE

Edizione completa

Medicinali e Tascabile

Medicinali

L'***unico*** prontuario ***completo*** e ***affidabile***

TUTTE LE INFORMAZIONI UTILI SEMPRE A PORTATA DI MANO

clienti.codifa@lswr.it
www.edizioniedra.it/if

edra

EDRA SpA
Tel. 02 88184 317 - 243
Via Spadolini, 7 - 20141 Milano

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

ITALY SUBSCRIPTION
only 60,00 Euro

INTERNATIONAL SUBSCRIPTION
only 85,00 Euro

SUBSCRIBE NOW!
www.eurannallergyimm.com

- 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 and case reports concerning etiology, diagnosis and treatment of allergic and immunological disorders
- includes a section of information coming from the main international health associations and authorities.

16045

To submit your paper go to http://eaaci.edmgr.com

EDRA Spa, Via Spadolini 7, 20141 Milano – Tel. 02 88 18 41 – abbonamentiedra@lswr.it