Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

Arch Bronconeumol. 2018;54(7):378–385

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

www.archbronconeumol.org

Special article

The MEGA Project: A Study of the Mechanisms Involved in the Genesis and Disease Course of Asthma. Asthma Cohort Creation and Long-Term Follow-Up☆

Xavier Muñoz,[a,b,c,*] María José Álvarez-Puebla,[d] Ebymar Arismendi,[b,e] Lourdes Arochena,[b,f] María del Pilar Ausín,[b,g] Pilar Barranco,[b,h] Irina Bobolea,[b,e] Jose Antonio Cañas,[b,f] Blanca Cardaba,[b,f] Astrid Crespo,[i] Victora del Pozo,[b,f] Javier Domínguez-Ortega,[b,h] María del Mar Fernandez-Nieto,[b,f] Jordi Giner,[i] Francisco Javier González-Barcala,[j] Juan Alberto Luna,[b,h] Joaquim Mullol,[b,e] Iñigo Ojanguren,[a,b] José María Olaguibel,[d] César Picado,[b,e] Vicente Plaza,[i] Santiago Quirce,[b,h] David Ramos,[i] Manuel Rial,[b,f] Christian Romero-Mesones,[a,b] Francisco Javier Salgado,[j] María Esther San-José,[j] Silvia Sánchez-Diez,[a,b] Beatriz Sastre,[b,f] Joaquin Sastre,[b,f] Lorena Soto,[i] Montserrat Torrejón,[i] Marisa Urnadoz,[d] Luis Valdes,[j] Antonio Valero,[b,e] María Jesús Cruz[a,b]

[a] *Servicio de Neumología, Hospital Vall d'Hebron, Barcelona, Spain*
[b] *CIBER de Enfermedades Respiratorias (CIBERES), Spain*
[c] *Departamento de Biología Celular, Fisiología e Inmunología, Universitat Autònoma de Barcelona, Barcelona, Spain*
[d] *Servicio de Alergología, Complejo Hospitalario de Navarra, Pamplona, Navarra, Spain*
[e] *Servicio de Neumología, Hospital Clínic, Universitat de Barcelona, IDIBAPS, Barcelona, Spain*
[f] *Departamento de inmunología, Servicio de Neumología, IIS-Fundación Jiménez Díaz, Madrid, Spain*
[g] *Servicio de Neumología, Hospital del Mar-IMIM, Barcelona, Spain*
[h] *Servicio de Alergia, Instituto de Investigación, Hospital Universitario La Paz (IdiPAZ), Madrid, Spain*
[i] *Departamento de Medicina Respiratoria, Hospital de la Santa Creu i Sant Pau, Instituto de investigación biomédica Sant Pau (IIB Snat Pau), Universidad Autonoma de Barcelona. Departamento de Medicina, Barcelona, Spain*
[j] *Servicio de Neumología, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, La Coruña, Spain*

ARTICLE INFO

Article history:
Received 23 October 2017
Accepted 20 December 2017
Available online 8 June 2018

Keywords:
Phenotype
Eosinophilic asthma
Neutrophilic asthma
Epigenetics
Exacerbations
Bronchiectasis
Fixed airflow obstruction
Biomarkers

ABSTRACT

The general aim of this study is to create a cohort of asthma patients with varying grades of severity in order to gain greater insight into the mechanisms underlying the genesis and course of this disease.

The specific objectives focus on various studies, including imaging, lung function, inflammation, and bronchial hyperresponsiveness, to determine the relevant events that characterize the asthma population, the long-term parameters that can determine changes in the severity of patients, and the treatments that influence disease progression. The study will also seek to identify the causes of exacerbations and how this affects the course of the disease.

Patients will be contacted via the outpatient clinics of the 8 participating institutions under the auspices of the Spanish Respiratory Diseases Networking System (CIBER). In the inclusion visit, a standardized clinical history will be obtained, a clinical examination, including blood pressure, body mass index, complete respiratory function tests, and FENO will be performed, and the Asthma Control Test (ACT), Morisky-Green test, Asthma Quality of Life Questionnaire (Mini AQLQ), the Sino-Nasal Outcome Test 22 (SNOT-22), and the Hospital Anxiety and Depression scale (HADS) will be administered. A specific electronic database has been designed for data collection. Exhaled breath condensate, urine and blood samples will also be collected. Non-specific bronchial hyperresponsiveness testing with methacholine will be performed and an induced sputum sample will be collected at the beginning of the study and every 24 months. A skin prick test for airborne allergens and a chest CT will be performed at the beginning of the study and repeated every 5 years.

© 2018 SEPAR. Published by Elsevier España, S.L.U. All rights reserved.

☆ Please cite this article as: Muñoz X, Álvarez-Puebla MJ, Arismendi E, Arochena L, del Pilar Ausín M, Barranco P, et al. Estudio de los mecanismos implicados en la génesis y evolución del asma (proyecto MEGA): creación y seguimiento a largo plazo de una cohorte de pacientes asmáticos. Arch Bronconeumol. 2018;54:378–385.

* Corresponding author.
E-mail address: xmunoz@vhebron.net (X. Muñoz).

1579-2129/© 2018 SEPAR. Published by Elsevier España, S.L.U. All rights reserved.

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

Palabras clave:
Fenotipo
Asma eosinofílica
Asma neutrofílica
Epigenética
Exacerbaciones
Bronquiectasias
Obstrucción fija
Biomarcadores

Estudio de los mecanismos implicados en la génesis y evolución del asma (proyecto MEGA): creación y seguimiento a largo plazo de una cohorte de pacientes asmáticos

R E S U M E N

El objetivo general del estudio es la creación de una cohorte de pacientes con asma con distintos grados de gravedad, que permita incrementar los conocimientos sobre los mecanismos subyacentes a la génesis y evolución de esta patología.
Los objetivos específicos se centran en llevar a cabo diferentes estudios en términos de imagen, de función pulmonar, inflamación e hiperrespuesta bronquial, para determinar los eventos relevantes que dan forma a esta población asmática, los parámetros a largo plazo que pueden determinar los cambios en la gravedad de los pacientes y que tratamientos pueden influir en la progresión de la enfermedad. El estudio también tratará de identificar las causas de las exacerbaciones y cómo esto afecta a la evolución de la enfermedad. Los pacientes serán contactados a través de las consultas externas de las 8 instituciones participantes en el marco del CIBER de Enfermedades Respiratorias. En la visita de inclusión, se realizará una historia clínica estandarizada, un examen clínico exhaustivo, incluyendo la presión arterial, el índice de masa corporal, las pruebas funcionales respiratorias completas y la medición de la FENO, y se administrarán los cuestionarios Test de control del asma (ACT), Morisky Green, Cuestionario de calidad de vida en pacientes con asma (Mini AQLQ), el Cuestionario sino-nasal Outcome Test 22 (SNOT-22) y la escala de ansiedad y depresión (HAD). Para la recogida de los datos se ha diseñado una base de datos electrónica específica. Se recogerán también muestras de aire exhalado condensado, orina y sangre. Al inicio del estudio y cada 24 meses, se realizará una prueba de hiperrespuesta bronquial inespecífica con metacolina y se recogerá una muestra de esputo inducido. Al inicio del estudio se realizarán prick test a neumoalérgenos y una tomografía computarizada torácica que se repetirá a los 5 años.
© 2018 SEPAR. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

Asthma is a global health problem. Prevalence of this disease has risen in recent decades, and it now thought to affect up to 358 million individuals worldwide. The problem is particularly significant in industrialized countries, which have witnessed a great increase in asthma rates in the last 50 years.[1,2] Asthma currently affects between 1% and 16% of the world population,[1] while prevalence in Spain ranges from 1.5% to 16.7% of the adult population and around 10% of children.[3] Asthma patients have a poorer quality of life, more work and school absenteeism, and a greater comorbidity burden. As this a chronic disease, socio-economic costs are high.[4]

Bronchial asthma is defined as an inflammatory disease of the airways that causes bronchial hyperresponsiveness or airflow obstruction, and which is manifested by symptoms such as cough, wheezing or dyspnea.[1,3] Asthma has traditionally been considered a disease associated with atopy or allergy with onset in childhood that can persist or resolve in adulthood.[5] However, it is now thought of as a heterogeneous and multifactorial disease with several phenotypes, each with its own natural history and distinct response to treatment.[6,7] In addition to allergic or extrinsic asthma and non-allergic or intrinsic asthma, other phenotypes have been defined in the last 20 years on the basis of clinical or physiological characteristics (severity, age at onset, grade of obstruction, resistance to treatment), triggering factors (exercise, allergens, occupation, aspirin-induced asthma), or type of inflammation (eosinophilic, neutrophilic or paucicellular).[8] These definitions focus on partial features of the disease, and while they may be helpful, they do not fully explain the complexities of asthma. Indeed, issues such as the causes of increasing asthma rates, genetic susceptibility, or the interaction between environmental factors and the immune system, both in the genesis of asthma or as a trigger of exacerbations, have yet to be resolved.

Two strategies have been developed in the past 10 years to improve understanding of this condition. The first is to classify asthma according to phenotypes, using cluster analysis.[9–11] Cluster analysis is based on multivariate mathematical algorithms that quantify similarities between individuals of the same population, in order to define the mechanisms of the disease and, thus, optimize treatment. The second strategy consists of the creation of different patient cohorts, in order to study the natural history of the disease. To date, 3 European cohorts,[12–14] and 1 American cohort[15] have been developed. The purpose of these cohorts is to optimize treatment according to the particular features of each patient. However, cohorts published to date focus on the study of severe or refractory asthma. To the best of our knowledge, no cohort studies have yet focused on the analysis of the overall asthma population. Moreover, none of these cohorts includes both lung function and inflammatory studies, nor has progressive bronchial imaging been collected.

This aim of this study, the MEGA project (*Me*chanism underlying the *g*enesis and evolution of *a*sthma), is to create a cohort that would give access to clinical, physiological, molecular, and genetic data in patients with varying degrees of asthma severity. The data collected will help establish the different physiopathological pathways that cause or impact on the heterogeneous expression of this disease, and will show what percentage of patients might progress to bronchiectasis or fixed bronchial obstruction, and identify factors that might promote or influence this progress. This is a multicenter study that will be performed in 8 hospitals, under the auspices of the Spanish Respiratory Disease Biomedical Research Network (CIBERES). Specific tests, including imaging, lung function, inflammation, and bronchial hyperresponsiveness, will be performed periodically to determine the relevant events that characterize the asthma population, the long-term parameters that can determine changes in severity, and the treatments that influence disease progression. The study, which is the first of its kind, will also seek to identify the causes of exacerbations and how they affect the course of the disease. In short, the aim is to improve our understanding of the natural history of the disease, without focusing exclusively on severe asthma (Fig. 1).

This article describes the cohort selection criteria, baseline characteristics and progress-related parameters that will be collected, as well as the methods used to obtain, process, and store the various biological samples in the CIBERES pulmonary biobank.

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

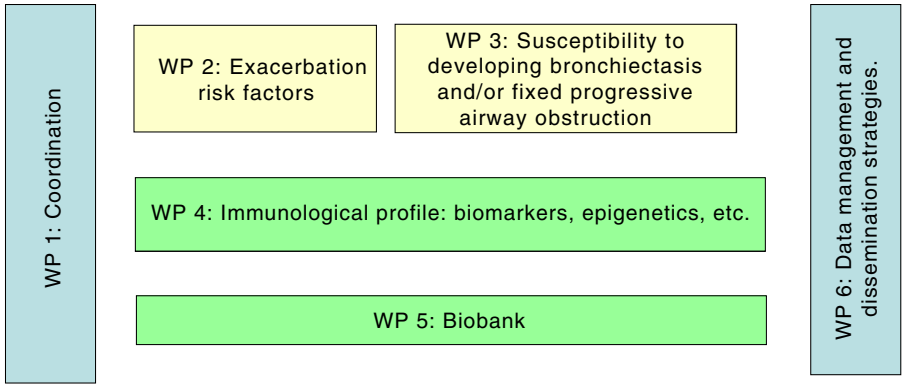

Fig. 1. Study design.

Methods

Design, Study Population and Clinical Variables

This is a prospective multicenter cohort study with an estimated representative sample of 525 adult patients with asthma and 100 healthy controls. Subjects will be recruited from 8 university hospitals in Spain.

Patient recruitment will be consecutive and non-selected, with an expected loss-to-follow-up rate of 12.5%. The first 75 patients with asthma seen during the study period in each hospital, aged 18–75 years, with intermittent, mild, moderate, or severe asthma, according to the GINA classification,[1] who have been diagnosed (also based on the GINA criteria) at least 1 year before inclusion, will be recruited. Patients will be excluded only if they have other acute or chronic active lung disorders, or significant psychiatric disorders. All patients will give informed consent in writing. Individuals will be contacted via the outpatient clinic of the participating institutions, and participants will be seen at the start of the study, every 6 months during the first year, and then every year. A control group of 100 healthy subjects, non-smokers, matched in age and gender with controls, will be recruited. The control group will undergo the same tests and evaluations as the asthma patients.

Common data collection methods will be used in all participating centers. At each visit, a standardized clinical history will be completed for each patient, and validated versions of the following questionnaires will be administered: Asthma Control Test (ACT),[16,17] Morisky Green,[18,19] Asthma Quality Of Life Questionnaire (20.21 Mini AQLQ),[20,21] Sino-Nasal Outcome Test 22 (22.23 SNOT-22)[22,23] and Hospital Anxiety and Depression (HAD).[24,25] A detailed clinical examination, including blood pressure, body mass index, full respiratory function tests, and the measurement of fractional exhaled nitric oxide (FeNO), will be performed. Exhaled breath condensate, urine and blood samples will also be collected. To ensure homogeneous sample collection, common protocols have been designed for use in all participating centers. Non-specific bronchial hyperresponsiveness testing with methacholine will be performed at the beginning of the study and every 24 months, and an induced sputum sample will be collected. Chest computed tomography (CT) and skin prick tests (SPT) for airborne allergens will be performed at the beginning of the study and every 5 years thereafter (Fig. 2).

Data Collection

A specific electronic database and case report form (CRF) has been designed to collect study data. This database will be used to collect, manage, and export previously anonymized data from the study patients. Database variables have been agreed by a multidisciplinary team of project investigators, and the CRF was designed by a specialized company (Biostatistics and Data Management Core Facility, IDIBAPS, Institut d'Investigacions Biomèdiques August Pi i Sunyer) (Annex 1, online supplement). The company responsible for CRF design and the project investigators will perform periodic reviews to ensure the quality of the data included in the database. These reviews will take place in the first month, and every six months thereafter.

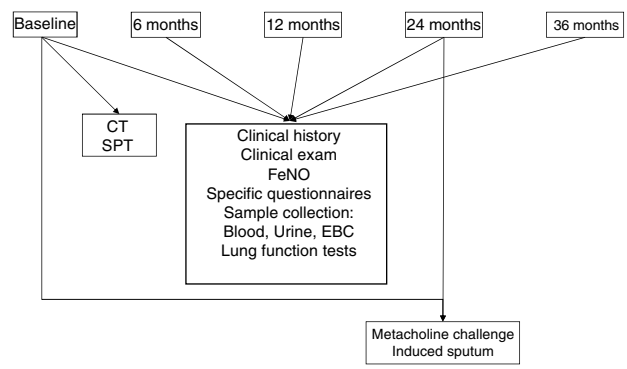

Fig. 2. Variables and samples collected in the different study phases. The study will run for at least 10 years. Chest CT will be performed at baseline and every 5 years. Methacholine challenge and induced sputum will be performed at baseline and every 2 years. CT: computed tomography; EBC: exhaled breath concentrate; SPT: skin prick tests.

Atopy

Atopy will be defined as the presence of at least 1 SPT or specific serum IgE positive for an airborne allergen.[26] SPTs will be performed for the most common airborne allergens in each study region, and must include: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*), pollens (*Cupressus arizónica*, *Platanus acerifolia*, *Olea europea*), grasses (*Artemisia vulgaris, Parietaria judaica, Salsola kali*), dander (cat, dog), molds (*Alternaria alternata*, *Aspergillus fumigatus*, *Caldosporium herbarum*, *Penicillium notatum*) and cockroach (*Blatella orientalis*). SPTs will be considered positive in the case of wheal of at least 3 mm in diameter compared to the negative control (saline); histamine 10 mg/ml will be used as a positive control.[26]

Serum specific IgE to common airborne allergens will be determined using a commercial kit (ImmunoCAP, Thermo Fisher

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

Scientific, Uppsala, Sweden), following the manufacturer's instructions. A result of at least 0.35 KU/l will be considered positive.

Lung Function Tests

Forced spirometry will be performed and analyzed according to the SEPAR guidelines.[27] The reference values proposed by Roca et al.[28] for the Mediterranean population will be used. Spirometry will be performed before the bronchodilator test, with the administration of 2 puffs of salbutamol through a spacer (0.1 mg β2-adrenergic agonist per inhalation), and 15 min after. The test will be considered positive when an increase in FEV1 or forced vital capacity (FVC) greater than 200 ml is recorded, representing more than 12% of the baseline value.[29] Static lung volumes will be determined by plethysmography and diffusing capacity for carbon monoxide (DLCO) will be tested using the single breath method. These studies will be conducted with MasterLab analyzer (MasterLab, Jaegger, Germany) in accordance with the proposed joint guidelines of the European Respiratory Society (ERS) and the American Thoracic Society (ATS).[30,31]

Bronchial hyperresponsiveness will be determined using a nonspecific methacholine bronchial challenge test according to the ERS/ATS guidelines.[32] The test will be considered positive if FEV1 decreases more than 20% from baseline. If this decrease is not observed after the administration of 5 puffs at a concentration of 16 mg/ml, the test will be considered negative.[33] The test result will be expressed as PC20 (concentration [mg/ml] of methacholine that provoked a 20% decrease in FEV1).

Determination of Fractional Exhaled Nitric Oxide

FeNO will be measured using a chemiluminescence analyzer (NiOx; Aerocrine; Solna, Sweden), according to the guidelines recently proposed by the ATS.[34]

Exhaled Breath Condensate

Exhaled breath condensate (EBC) will be collected using an EcoScreen device (Erich Jaegger GmbH, Würzburg, Germany), following ERS/ATS guidelines.[35] Briefly, patients will be advised not to eat or drink anything for 1 h before the test. They must then breathe tidally while connected to the device until a total of 150 L of exhaled air has been collected. When the sample has been collected, pH will then be measured after bubbling helium through the EBC for 10 min. Aliquots of 0.5 ml will be prepared and stored at −80 °C for future biomarker studies.

Induced Sputum

Sputum will be induced by inhaling increasing concentrations of hypertonic saline (3%, 4%, and 5%) for 7 min. It will be processed and examined as previously described by Pizzichini et al.[36] The quantitative cell count of all cells and different cell types will be determined in all sputum samples. Supernatant will be frozen at −80 °C.

Blood and Urine Samples

All individuals will provide a sample of whole blood for DNA extraction (10 ml), plasma (10 ml), and urine. These samples will be retained for future genetic and molecular studies.

Computed Tomography

All individuals included in the study will undergo a chest CT at the start of the study and then every 5 years. This study will be performed to determine the presence of bronchiolectasis (bronchus/artery ratio >1), bronchiectasis (bronchus/artery ratio >1.5), and bronchi with thickened walls, and the bronchial wall of the apical segment of the right upper lobe near its origin (mm) will be measured.[37]

Storage of Samples

Serum, DNA, EBC, and sputum supernatant will be stored at −80 °C in each of the recruiting centers. One of the objectives of creating this cohort is to develop a specimen biobank. These samples will be included in the already existing CIBERES biobank, where they will be stored for future use in biomarker studies.

Ethical and Legal Considerations

This study has been designed according to the principles of the Declaration of Helsinki (18th World Medical Assembly, 1964) and Hong Kong (1989). The purpose of the study will be explained to each patient before their consent to participate is requested. They will receive a letter explaining the utility and clinical interest of the study, and the potential risks of participating. The research project has been approved by the Clinical Research Ethics Committee of all hospitals participating in the study in accordance with Personal Data Protection Act 15/1999, Biomedical Research Act 14/2007, and Biomedical Research Royal Decree 1716/2011.

Discussion

The main aim of creating this cohort is to contribute to the understanding of the heterogeneity of asthma. Accordingly, we will perform immunological studies to clarify the mechanisms involved in the development of differing clinical manifestations among patients, epigenetic studies to identify the factors leading to the appearance of asthma, studies to identify risk factors for exacerbations, and studies to identify patients who are susceptible to developing bronchiectasis and irreversible progressive airway obstruction.

A large proportion of current research in asthma is aimed at identifying the immunological pathways that determine disease, including disease phenotypes and endotypes and certain biomarkers that may help us design precision medicine.[38] It is generally agreed that 3 possible immune responses can be distinguished in the development of asthma, namely type 2 immune response, non-type 2 immune response, and mixed type Th2/Th17 immune response,[39] but the intimate mechanisms that generate these responses are still largely unknown. Type 2 response is the best known, and is generally observed in asthma patients with eosinophilic inflammation.[40] The distinctive feature of this response is a Th2 pathway which leads to the final production of specific IgE, the basic effector cells of which appear to be Th2 lymphocytes and plasma cells. The main cytokines involved are IL-4, IL-5 and IL-13.[41] The type 2 response also occurs in non-atopic patients, apparently driven by innate T lymphocyte (ILC2), with IL-5 as the main effector cytokine.[42] Before differentiation, both pathways appear to have common bronchial epithelium-derived cytokines, such as IL-33, IL-31, IL-25 and thymic stromal lymphopoietin, and these molecules are currently under investigation as possible therapeutic targets[43–45] (Fig. 3). It is unclear why this second pathway, with its low Th2 profile but marked eosinophilic inflammation, is associated with more severe asthma and a poorer response to corticosteroids.

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

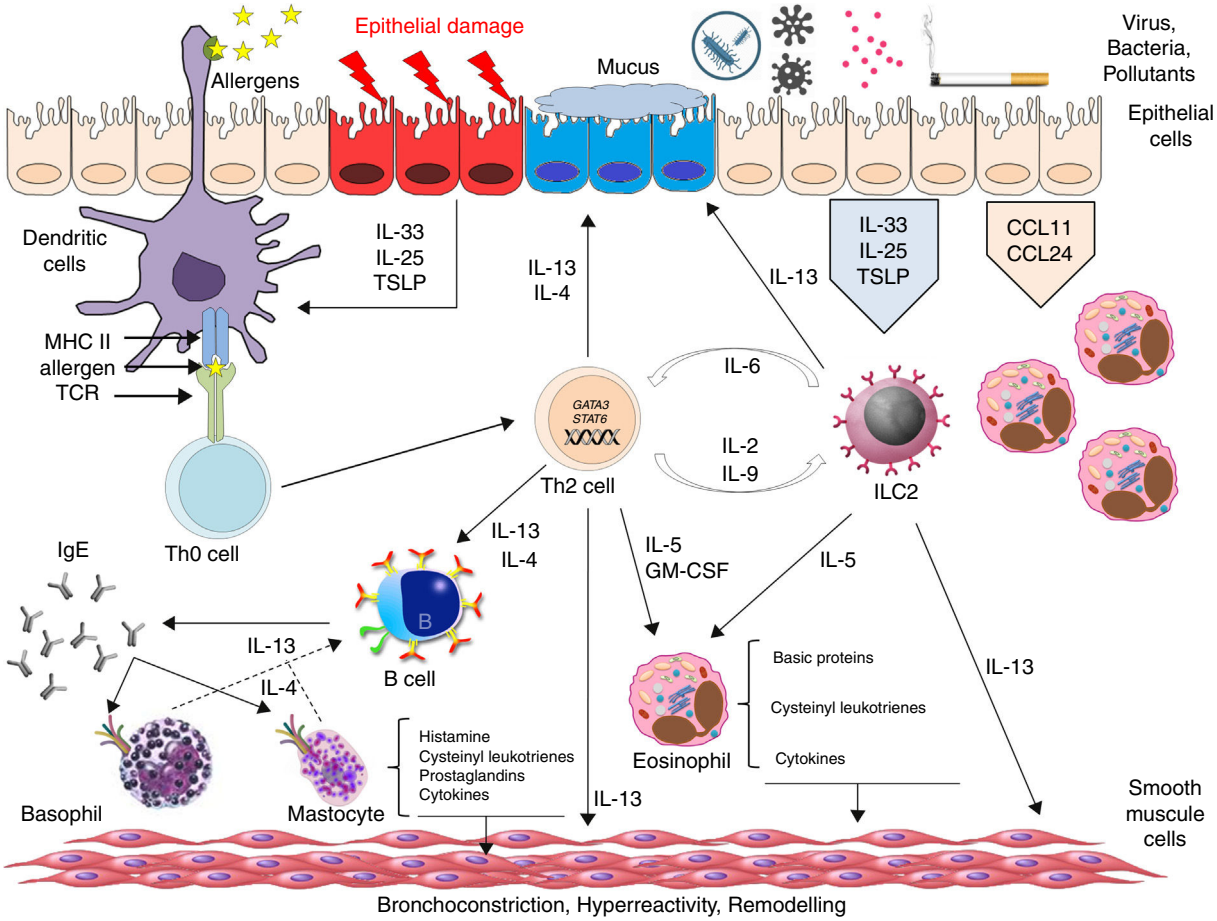

Fig. 3. Main pathways involved in type 2 immune response in bronchial asthma © 2018 Xavier Muñoz Gall.

Non-type 2 immune response, also known as non-eosinophilic asthma,[10,46] appears to include patients with Th17-dependent neutrophilic inflammation,[47,48] patients with neutrophilic inflammation dependent on dysregulation of the innate immune response associated with IL-1b or CXCR2,[49] and patients with neurogenic inflammation basically associated with the RTPA1 receptors.[50,51] While these response types are the focus of several studies, the putative role of tumor necrosis factor-alpha, IL-6, IL-8, IL-37, IL-22 and IL-9 is under discussion.[39]

Although patients with a mixed Th17/type 2 response have been described, the relationship between the 2 response pathways is highly complex and little understood. IL-17 produced by T cells or by the Th17 cells themselves in response to possible epithelial damage was seen to increase the production of IL-4 and IL-13 by ILC2 and Th2 cells in an atopic dermatitis animal model.[52] At the same time, IL-4 and IL-13 seem to be capable of amplifying the Th17 response by regulating CD209a expression in dendritic cells in murine schistosomiasis models.[53] However, in murine asthma models, the neutralization of IL-4 or IL-13 results in an increase in Th17 cells and neutrophilic inflammation, while the neutralization of IL-13 and IL-17 prevents both eosinophilic and neutrophilic inflammation and the appearance of bronchial hyperresponsiveness.[54] A better understanding of these pathways and their possible interactions obtained from the possible results of this cohort, may translate to a better selection of therapeutic targets and the implementation of combined treatments, such as double Th2/Th17 blockade.

Asthma is clearly a complex disease in which both genetic and environmental factors play a decisive role. However, neither genetic[55] nor environmental[56] factors alone can explain the variability observed in these patients. Epigenetic studies may help determine genetic-environmental interactions and may shed light on the hereditary component of asthma.[57,58] Epigenetic mechanisms are known to regulate the expression of cytokines that play an important role in the differentiation of T lymphocytes and certain transcription factors,[59,60] which in turn may be influenced by different environmental exposures.[61] Indeed, exposure to diesel particulate matter has been associated with DNA methylation in peripheral blood and in the epithelial cells of the airways, and with altered T reg cell function when associated with exposure to polycyclic aromatic hydrocarbons.[62,63] Although exposure to certain allergens has also been associated with methylation changes in the DNA of respiratory epithelial cells,[62] very few studies have evaluated the influence of multiple exposures on epigenetic changes.

The interaction between changes in the microbiome and epigenetic changes is another interesting area. Epidemiological studies have described a lower incidence of asthma in individuals with less exposure to antibiotics in early life,[64] and in babies delivered vaginally rather than by Cesarean section.[65] In fact, there is a strong relationship between exposure to microorganisms and susceptibility to asthma in early life. Several authors believe that these early exposures may affect the composition of the microbiome of the individual and this, in turn, may generate epigenetic changes that will lead to a greater susceptibility to developing asthma.[58]

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

Although we have no plans to develop microbiome studies in this cohort, we will take into account these multiple factors that may determine epigenetic changes and, as such, may be responsible for the heterogeneity of asthma.

Little is understood about the factors that determine exacerbations in asthmatic patients. Various factors have been implicated, including respiratory infections, especially of viral origin,[66–68] exposure to allergens,[69] smoking,[70] lack of adherence to treatment,[71] rhinosinusitis,[72,73] obesity, intolerance of non-steroidal anti-inflammatory drugs,[74] and psychosocial factors.[75] However, allergic sensitization is known to vary widely, depending on environmental and climatic conditions.[76] Moreover, while viral infection seems to be a major trigger for exacerbations,[66–68] the interaction between these infections and allergic sensitization associated with a specific bronchial inflammatory response has not been studied in depth, nor are studies available that associate certain factors of exacerbation with different asthma phenotypes.

While a relationship between the degree of control of asthma and the risk of exacerbations has been established,[77] evidence that they are not always associated[78] is supported by the fact that inflammation and bronchial hyperresponsiveness persist in patients with controlled asthma.[79] It is difficult to achieve disease control in patients with severe asthma, and treatment is essentially aimed at avoiding exacerbations.[3] Several markers have been proposed in an attempt to establish the risk of exacerbation, such as FEV1 or the number of exacerbations in the previous year.[80] A clinical index for predicting exacerbations has recently been developed[81]; however, it was created from retrospective analyses and has not been fully validated, nor does it include measurements of inflammatory factors, such as FeNO or eosinophils in induced sputum, that are essential data in this disease.

Finally, some asthma patients are known to develop bronchiectasis,[82] while others develop fixed airway obstruction practically indistinguishable from that experienced by patients with chronic obstructive pulmonary disease.[83] Both factors predict a worse prognosis and a greater use of healthcare resources.[84] These events have conventionally been associated with patients with more severe asthma or poorer disease control and a greater number of exacerbations[85,86]; however, the factors that determine which patients with similar clinical characteristics will go on to develop these complications remain unclear. Nor do we know if this poor prognosis can occur in less severe forms of the disease.

In conclusion, this large Spanish, prospective, multidisciplinary cohort has been created to clarify the molecular mechanisms that influence the different clinical presentations of the disease and the factors associated with certain mechanisms that may affect exacerbations or the appearance of bronchial changes in asthma patients, in the form of bronchiectasis or fixed progressive airway obstruction. Another aim is to identify potentially preventable epigenetic changes and to try to discover biomarkers to help determine the most effective treatment for each patient.

Funding

This study is funded by FIS PI15/0190 (Instituto de Salud Carlos III), the European Regional Development Fund (ERDF) and Sanofi. MJC receives funding from the Miguel Servet research program of the Instituto de Salud Carlos III (CP12/03101).

Authorship

All authors of the manuscript have significantly contributed to the research, preparation, review, and final version of the manuscript.

Conflict of Interest

The authors state that they have no conflict of interests.

Appendix A. Supplementary Data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.arbr.2018.05.011.

References

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www.ginasthma.org [accessed 18.10.17].
2. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5:691–706.
3. Guía española para el manejo del asma (GEMA 4.2) 2017. Available from: www.gemasma.com [accessed 18.10.17].
4. Uphoff E, Cabieses B, Pinart M, Valdés M, Antó JM, Wright J. A systematic review of socioeconomic position in relation to asthma and allergic diseases. Eur Respir J. 2015;46:364–74.
5. Koh MS, Irving LB. The natural history of asthma from childhood to adulthood. Int J Clin Pract. 2007;61:1371–4.
6. Borish L, Culp JA. Asthma: a syndrome composed of heterogeneous diseases. Ann Allergy Asthma Immunol. 2008;101:1–8.
7. Szefler SJ. Defining asthma phenotipes: focusing the picture. J Allergy Clin Immunol. 2010;126:926–38.
8. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet. 2006;368:804–13.
9. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178:218–24.
10. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al., National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181:315–23.
11. Siroux V, Basagaña X, Boudier A, Pin I, Garcia-Aymerich J, Vesin A, et al. Identifying adult asthma phenotypes using a clustering approach. Eur Respir J. 2011;38:310–7.
12. The ENFUMOSA study group. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. Eur Respir J. 2003;22:470–7.
13. Kupczyk M, Wenzel S. US and European severe asthma cohorts: what can they teach us about severe asthma? J Int Med. 2012;272:121–32.
14. Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. Eur Respir J. 2015;46:1227–31.
15. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al., National Heart, Lung, Blood Institute's Severe Asthma Research Program. Characterization of severe asthma phenotype by the National Heart, Lung and Blood Institute's Severe asthma Research Program. J Allergy Clin Immunol. 2007;119:405–13.
16. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004;113:59–65.
17. Covalair Investigator GroupVega JM, Badia X, Badiola C, López-Viña A, Olaguíbel JM, Picado C, et al. Validation of the Spanish version of the Asthma Control Test (ACT). J Asthma. 2007;44:867–72.
18. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986;24:67–74.
19. Val Jiménez A, Amorós Ballestero G, Martínez Visa P, Fernández Ferré ML, León Sanromà M. Descriptive study of patient compliance in pharmacologic antihypertensive treatment and validation of the Morisky and Green test. Aten Primaria. 1992;10:767–70.
20. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J. 1999;14:32–8.
21. Perpiñá M, Belloch A, Pascual LM, de Diego A, Compte L. Calidad de vida en el asma: validación del cuestionario AQLQ para su utilización en población española. Arch Bronconeumol. 1995;31:211–8.
22. Baumann I. Subjective outcomes assessment in chronic rhinosinusitis. Open Otorhinolaryngolog J. 2010;4:28–33.
23. De los Santos G, Reyes P, del Castillo R, Fragola C, Royuela A. Cross-cultural adaptation and validation of the sino-nasal outcome test (SNOT-22) for Spanish-speaking patients. Eur Arch Otorhinolaryngol. 2015;272:3335–40.
24. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67:361–70.

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

25. Terol MC, López-Roig S, Rodríguez-Marín J, Martín-Aragón M, Pastor MA, Reig MT. Propiedades psicométricas de la Escala hospitalaria de ansiedad y estrés (HAD) en población española. Ansiedad y Estrés. 2007;13:163–76.
26. Dreborg ES, Frew A, Frew A. Position paper: allergen standardization and skin tests. Allergy. 1993;48:48–82.
27. García-Río F, Calle M, Burgos F, Casan P, del Campo F, Galdiz JB, et al. Espirometría. Arch Bronconeumol. 2013;49:388–401.
28. Roca J, Sanchis J, Agusti-Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, et al. Spirometric references values from a Mediterranean population. Bull Eur Physiophatol Respir. 1986;22:217–24.
29. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis. 1991;144:1202–18.
30. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26: 511–22.
31. MacIntyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J. 2005;26:720–35.
32. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American thoracic society was adopted by the ATS board of Directors, July 1999. Am J Respir Crit Care Med. 2000;161: 309–29.
33. Nair P, Martin JG, Crockcroft DC, Dolovich M, Lemiere C, Boulet LP, et al. Airway hyperresponsiveness in asthma: measurement and clinical relevance. J Allergy Clin Immunol Pract. 2017;5:649–59, e2.
34. American Thoracic Society. Recommendation for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Am J Respir Crit Care Med. 1999;160:2104–17.
35. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005;26:523–48.
36. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Resp Crit Care Med. 1996;154:308–17.
37. Smith IE, Jurriaans E, Diederich S, Ali N, Shneerson J, Flower CRD. Chronic sputum production: correlation between clinical features and findings on high resolution computed tomographic scanning of the chest. Thorax. 1996;51: 914–8.
38. Murano A, Lemanske RF, Hellings PW, Akdis CA, Bieber T, Casale TB, et al. Precision medicine in patients with allergic disease: airway diseases and atopic dermatitis-PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2016;137:1347–58.
39. Agache I, Akdis CA. Endotypes of allergic diseases and asthma: an important step in building blocks for the future of precision medicine. Allergol Int. 2016;65:243–52.
40. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: eosinophilic airway inflammation in nonallergic asthma. Nat Med. 2013;19:977–9.
41. Pavord ID, Afzalnia S, Menzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clin Exp Allergy. 2017;47:148–60.
42. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol. 2004;113:101–8.
43. Nabe T. Interleukin (IL)-33: new therapeutic target for atopic diseases. J Pharmacol Sci. 2014;126:85–91.
44. Martínez-González I, Cruz MJ, Moreno R, Morell F, Muñoz X, Aran JM. Human mesenchymal stem cells resolve airway inflammation, hyperreactivity and histopathology in an occupational asthma model. Stem Cell Dev. 2014;23:2352–63.
45. Ojanguren I, Martin JG, Lemiere C. Thymic stromal lymphopoietin: a promising target in the treatment of asthma? Arch Bronconeumol. 2017;53:545–6.
46. Agache I. Non-eosinophilic asthma endotypes. Curr Treat Options Allergy. 2015;2:257–67.
47. Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol. 2009;123:986–94.
48. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. Am J Respir Crit Care Med. 2009;180:720–30.
49. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. J Allergy Clin Immunol. 2014;133:997–1007.
50. Butler CA, Heaney LG. Neurogenic inflammation and asthma. Inflamm Allergy Drug Targets. 2007;6:127–32.
51. Yang H, Li S. Transient receptor potential ankyrin 1 (TRPA1) channel and neurogenic inflammation in pathogenesis of asthma. Med Sci Monit. 2016;22:2917–23.
52. Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. J Invest Dermatol. 2014;34:2122–30.
53. Ponichtera HE, Shinheat MG, Liu BC, Raychowdhury R, Larkin BM, Russo JM, et al. CD209a expression on dendritic cells is critical for the development of pathogenic Th17 cell responses in murine schistosomiasis. J Immunol. 2014;192:4655–65.
54. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med. 2015;7, 301ra129.
55. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet. 2015;386:1075–85.
56. Weichenthal S, Bai L, Hatzopoulou M, van Ryswyk K, Kwong JC, Jerrett M, et al. Long-term exposure to ambient ultrafine particles and respiratory disease incidence in Toronto, Canada: a cohort study. Environ Health. 2017;16: 64.
57. Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. J Allergy Clin Immunol. 2017;140:1–12.
58. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. J Allergy Clin Immunol. 2017;140:14–23.
59. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulations by cis elements, and epigenetics. Immunity. 2006;24: 369–79.
60. Jones B, Chen J. Inhibition of IFN-gamma transcription by site-specific methylation during T helper cell development. EMBO J. 2006;25:243–52.
61. Yang IV, Schwartz DA. Epigenetic mechanisms and the development of asthma. J Allergy Clin Immunol. 2012;130:1243–55.
62. Clifford RL, Jones MJ, MacIsaac JL, McEwen LM, Goodman SJ, Mostafavi S, et al. Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA mutilation. J Allergy Clin Immunol. 2017;139:112–21.
63. Hew KM, Walker AT, Kohli A, Garcia M, Syed A, McDonald-Hyman C, et al. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin Exp Allergy. 2015;45:238–48.
64. Kuo CH, Kuo HF, Huang CH, Yang SN, Lee MS, Hung CH. Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis. J Microbiol Immunol Infect. 2013;46: 320–9.
65. Tollancs MC, Moster D, Daltveit AK, Irgens ML. Cesarean section and risk of severe childhood asthma: a population based cohort study. J Pediatr. 2008;153: 112–6.
66. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. BMJ. 1995;310:1225–9.
67. Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol. 2004;114:239–47.
68. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ. 1993;307:982–6.
69. Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G, Phipatanakul W. Indoor environmental exposures and exacerbation of asthma: an update to the 2000 review by the Institute of Medicine. Environ Health Perspect. 2015;123:6–20.
70. Silverman RA, Hasegawa K, Egan DJ, Stiffler KA, Sullivan AF, Camargo CA Jr. Multicenter study of cigarette smoking among adults with asthma exacerbations in the emergency department, 2011–2012. Respir Med. 2017;125:89–91.
71. Wu AC, Butler MG, Li L, Fung V, Kharbanda EO, Larkin EK, et al. Primary adherence to controller medications for asthma is poor. Ann Am Thorac Soc. 2015;12:161–6.
72. Bousquet J, Schunemann HJ, Samolinski B, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic rhinitis and its impact on asthma (ARIA): achievements in 10 years and future needs. J Allergy Clin Immunol. 2012;130:1049–62.
73. Schatz M, Zeiger RS, Zhang F, Chen W, Yang SJ, Camargo CA Jr. Overweight/obesity and risk of seasonal asthma exacerbations. J Allergy Clin Immunol Pract. 2013;1:618–22.
74. Ledford DK, Wenzel SE, Lockey RF. Aspirin or other nonsteroidal inflammatory agent exacerbated asthma. J Allergy Clin Immunol Pract. 2014;2:653–7.
75. Powell H, McCaffery K, Murphy VE, Hensley MJ, Clifton VL, Giles W, et al. Psychosocial variables are related to future exacerbation risk and perinatal outcomes in pregnant women with asthma. J Asthma. 2013;50:383–9.
76. Baldacci S, Maio S, Cerrai S, Sarno G, Baïz N, Simoni M, et al., HEALS Study. Allergy and asthma: effects of the exposure to particulate matter and biological allergens. Respir Med. 2015;109:1089–104.
77. Brisk R, Heaney LG. Asthma control and exacerbations: two different sides of the same coin. Curr Opin Pulm Med. 2016;22:32–7.
78. Patel M, Pilcher J, Reddel HK, Qi V, Mackey B, Tranquilino T, et al. Predictors of severe exacerbations, poor asthma control, and β-agonist overuse for patients with asthma. J Allergy Clin Immunol Pract. 2014;2:751–8.
79. Muñoz X, Sanchez-Vidaurre S, Roca O, Torres F, Morell F, Cruz MJ. Bronchial inflammation and hyperresponsiveness in well-controlled mild or moderate asthma. Clin Exp Allergy. 2012;42:1321–8.
80. Gonzalez-Barcala FJ, Calvo-Alvarez U, Garcia-Sanz MT, Garcia-Couceiro N, Martin-Lancharro P, Pose A, et al. Asthma exacerbations: risk factors for hospital readmissions. Ir J Med Sci. 2018;187:155–61.
81. Bateman ED, Buhl R, O'Byrne PM, Humbert M, Reddel HK, Sears MR, et al. Development and validation of a novel risk score for asthma exacerbations: the risk score for exacerbations. J Allergy Clin Immunol. 2015;135:1457–64, e4.
82. Lujan M, Gallardo X, Amengual MJ, Bosque M, Mirapeix RM, Domingo C. Prevalence of bronchiectasis in asthma according to oral steroid

Document downloaded from http://www.elsevier.es, day 05/01/2019. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.

requirement: influence of immunoglobulin levels. Biomed Res Int. 2013;2013:109219.
83. Bujarski S, Parulekar AD, Sharafkhaneh A, Hanania NA. The asthma COPD overlap syndrome (ACOS). Curr Allergy Asthma Rep. 2015;15:509.
84. Hardin M. The clinical features of the overlap between COPD and asthma. Respir Res. 2011;12:127.
85. Dimakou K, Gousiou A, Toumbis M, Kaponi M, Chryssikos S, Thanos L, et al. Investigation of bronchiectasis in severe uncontrolled asthma. Clin Respir J. 2017, http://dx.doi.org/10.1111/crj.12653 [Epub ahead of print].
86. Oguzulgen IK, Kervan F, Ozis T, Turktas H. The impact of bronchiectasis in clinical presentation of asthma. South Med J. 2007;100:468–71.