Universitat Autònoma de Barcelona

ASTHMA AND BRONCHIECTASIS: GENERAL CHARACTERISTICS, CLINICAL IMPACT, AND INFLAMMATORY PROFILE

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DOCTORAL THESIS

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LIST OF ABBREVIATIONS

A

ABPA Allergic Bronchopulmonary Aspergillosis

ACT Asthma Control Test

ACQ Asthma Control Questionnaire

AHR Airway Hyperresponsiveness

APC Antigen-Presenting Cells

B

BAL Bronchoalveolar Lavage

BMI Body Mass Index

BE Non-Cystic Fibrosis Bronchiectasis

C

CD Cluster of Differentiation

COPD Chronic Obstructive Pulmonary Disease

CXCR2 Interleukin 8 receptor beta

D

DC Dendritic Cells

DNA Deoxyribonucleic Acid

DLCO Diffusing Capacity of Lung for Carbon Monoxide

DTT Dithiothreitol

E

ECP Eosinophil Cationic Protein

ELISA Enzyme-Linked Immune Sorbent Assay

EMBARC The European Bronchiectasis Registry

F

FeNO Fractional Concentration of Exhaled Nitric Oxide

FEV1 Forced Expiratory Volume in one second

FVC Forced Vital Capacity

G

GEMA Spanish Guideline on the Management of Asthma

GINA Global Initiative for Asthma

GM-CSF Granulocyte-Macrophage Colony-Stimulating Factor

H

HDM House Dust Mites

HADs Hospital Anxiety and Depression Scale

HRCT High-Resolution Computed Tomography

I

ICS Inhaled corticosteroids

IFNγ Interferon-gamma

IL Interleukins

K

KCO Carbon Monoxide Transfer Coefficient

L

LABA Long-acting β2 agonists

LTRA Leukotriene Receptor Antagonists

M

MHC Major Histocompatibility Complex

MiniAQLQ Mini Asthma Quality of Life Questionnaire

MPPs Microorganisms with Potential Pathogenicity

MBP Major Basic Protein

N

NICE National Institute for Health and Care Excellence Guidelines

NIH National Institutes of Health

NSAIDs Non-steroidal anti-inflammatory drugs

O

OCS Oral Corticosteroids

P

PBS Phosphate Buffered Saline

PEF Peak Expiratory Flow

R

RADS Reactive Airways Dysfunction Syndrome

RV Residual Volume

S

SABA Short-acting β agonists

SARP Severe Asthma Research Program

SIC Specific Inhalation Challenge

SLPI Secretory Leukocyte Protease Inhibitor

T

Th T helper cells

TGF-β1 Transforming Growth Factor beta1

TLC Total Lung Capacity

TNF-α Tumour Necrosis Factor Alpha

TIMP Tissue Inhibitor of Matrix Metalloproteinase

V

VEGF Vascular Endothelial Growth Factor

W

WEA Work-Exacerbated Asthma

WRA Work-Related Asthma

INDEX

SUMMARY

Asthma is a heterogeneous airway disease with multiple phenotypes. Severe asthma is in only a minority of patients, but this patient cohort present relevant comorbidities and represent a large socioeconomic burden. Bronchiectasis is one of the most frequent comorbidities in severe asthma, characterized by pathological dilation of the airways. The presence of bronchiectasis may mimic asthma symptoms, resulting in poor asthma control with persistent clinical symptoms and difficult disease management. The mechanisms through which asthma promotes the evolution and development of bronchiectasis remain obscure. Moreover, it is uncertain whether a cause-effect relationship exists between asthma and bronchiectasis, but there is no doubt that asthma and bronchiectasis share some common features with respect to the innate immune activation and airway remodelling. Although in bronchiectasis the role of microorganism infection predominates while the pathogenesis of asthma is led mainly by the different adaptive immune responses, in many cases it is difficult to identify which one is the underlying condition.

Identifying bronchiectasis in severe asthma patients and achieving a better understanding of the inflammatory phenotypes and the underlying mechanism is therefore of key importance in precision medicine.

The primary objective of the present thesis is to study the inflammatory phenotypes of asthma patients with bronchiectasis. We also aim to quantify the expression of proinflammatory and remodelling cytokines in asthma patients with and without bronchiectasis, to assess the prevalence of bronchiectasis in patients with severe asthma, and to study the impact of bronchiectasis on the clinical manifestation of asthma.

Two studies were designed to address the above issues. In the first study, 224 moderate and severe asthma patients attended at our specialist asthma unit in 2018 were included. Features of bronchiectasis were assessed by Reiff and FACED parameters. Logistic regression was used to identify independent factors associated with bronchiectasis. In the second study, patients with severe asthma were recruited to perform sputum induction. Finally, severe asthma patients with bronchiectasis (AB, n=55), and patients with isolated asthma as controls (AC, n=45) were included. In the induced sputum samples, differential cell counts were performed, and proinflammatory and bronchial remodelling cytokines were analysed by immunoassay (IL8, neutrophilic elastase, TGFβ1, VEGF, IFNγ, TNFα, and GM-CSF).

In summary, bronchiectasis was identified in 78 patients, with a prevalence of 56.9% in severe asthma patients. Of all those with bronchiectasis, 81% were classified as mild bronchiectasis using modified Reiff criteria and 74% using FACED criteria. Asthma patients with bronchiectasis had decreasing FEV1, FVC and FEV1/FVC ($p = 0.002$, 0.005 and 0.014 respectively), presented more frequent asthma exacerbations ($p < 0.001$) and worse asthma control (ACT 21 vs 16pts, $p<0.001$). Asthma patients with bronchiectasis also suffered more sinusitis (10.3% vs 2.1%, $p =0.018$) and nasal polyps (28.2% vs 13.0%, $p=0.005$), but less atopic asthma (43.6% vs 63.0%, $p=0.005$) than those without bronchiectasis. Factors independently associated with bronchiectasis were older age (42-65 years: OR, 3.99; 95% CI, 1.60 to 9.95, $P = 0.003$; ≥ 65years: OR, 2.91; 95% CI, 1.06 to 8.04, $P = 0.039$), severe asthma grade (OR, 8.91; 95% CI, 3.69 to 21.49; $P <0.001$) and asthma exacerbations (OR, 4.43; 95% CI, 1.78 to 11.05; $P <0.001$). In patients with severe asthma, age of asthma onset (OR, 1.02; 95% CI, 1.01 to 1.04; $P =0.015$) and asthma exacerbations (OR, 4.88; 95% CI, 1.98 to 12.03; $P =0.001$) were independently associated with the development of bronchiectasis.

With regard to the inflammatory phenotype, neutrophilic inflammation was the primary phenotype in both severe asthma groups. Almost 80% of patients presented granulocytic inflammation (eosinophilic, neutrophilic or mixed). Higher levels of TGFβ1, VEGF and IFNγ were observed in asthma patients with bronchiectasis (AB) than in controls (AC) (15 vs 24 pg / ml, $p = 0.014$; 183 vs 272 pg / ml, $p = 0.048$; 0.85 vs 19 pg / ml, $p <0.001$ respectively). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was significantly decreased in group AB (1.2 vs. 4.4 pg / ml, $p <0.001$). IL-8, neutrophil elastase and TNFα did not present significant differences between the groups.

In conclusion, the prevalence of bronchiectasis is high in asthma patients, especially in those with severe asthma. In these patients, age of asthma onset and exacerbations were independent factors associated with the occurrence of bronchiectasis. The type of inflammation in asthma patients did not differ according to the presence or absence of bronchiectasis. Airway remodelling activation was observed, with increased levels of transforming growth factor beta1 (TGFβ1) and vascular endothelial growth factor (VEGF) cytokines in asthma patients with bronchiectasis.

KEYWORDS: asthma; bronchiectasis; inflammation; cytokines

RESUMEN

El asma es una enfermedad respiratoria heterogénea con múltiples fenotipos. El asma grave se presenta solo en una minoría de pacientes, pero estos pacientes presentan comorbilidades relevantes y representan una gran carga socioeconómica. Las bronquiectasias son una de las comorbilidades más frecuentes en el asma grave, y están caracterizadas por la dilatación patológica de las vías respiratorias. La presencia de bronquiectasias puede imitar los síntomas del asma, lo que resulta en un control deficiente del asma, con síntomas clínicos persistentes y un manejo difícil de la enfermedad. Los mecanismos a través de los cuales el asma promueve la evolución y el desarrollo de las bronquiectasias siguen aún en estudio. Además, no está claro si existe una relación causa-efecto entre el asma y las bronquiectasias, pero no hay duda de que el asma y las bronquiectasias comparten algunas características comunes con respecto a la activación de la respuesta inmune innata y el remodelado de las vías respiratorias. Aunque en las bronquiectasias predomina el papel de la infección por microorganismos, mientras que la patogénesis del asma está dirigida principalmente por las diferentes respuestas inmunes adaptativas, en muchos casos es difícil identificar cuál es la condición subyacente.

Por lo tanto, identificar la presencia de bronquiectasias en pacientes con asma grave y lograr una mejor comprensión de los fenotipos inflamatorios y el mecanismo subyacente es de importancia clave en la medicina de precisión.

El objetivo principal de la presente tesis es estudiar los fenotipos inflamatorios de pacientes con asma y bronquiectasias. También pretendemos cuantificar la expresión de citocinas proinflamatorias y de remodelado en pacientes con asma (con y sin bronquiectasias), evaluar la prevalencia de bronquiectasias en pacientes con asma grave y estudiar el impacto de las bronquiectasias en la manifestación clínica del asma.

Se diseñaron dos estudios para abordar los problemas anteriores. En el primer estudio, se incluyeron 224 pacientes con asma moderada y grave atendidos en una unidad especializada de asma en 2018. Las características de las bronquiectasias se evaluaron mediante los parámetros Reiff y FACED. Se utilizaron análisis de regresión logística para identificar factores independientes asociados con la presencia de bronquiectasias. En el segundo estudio, se seleccionaron los pacientes con asma grave, para analizar biomarcadores en esputo inducido. Se incluyeron pacientes con asma grave y bronquiectasias (AB, n = 55) y pacientes con asma sin bronquiectasias como controles (AC, n = 45). En las muestras de esputo inducido, se realizaron recuentos diferenciales

de células y se analizaron, mediante inmunoensayos específicos, los niveles de citocinas proinflamatorias y de remodelado bronquial (IL8, elastasa neutrofílica, TGFβ1, VEGF, IFNγ, TNFα y GM-CSF).

En resumen, se identificó la presencia de bronquiectasias en 78 pacientes, con una prevalencia del 56,9%, en pacientes con asma grave. En los pacientes con bronquiectasias, el 81% se clasificaron como bronquiectasias leves utilizando criterios Reiff modificados y el 74% utilizando criterios FACED. Los pacientes con asma y bronquiectasias presentaban unos valores de FEV1, FVC y FEV1 / FVC inferiores en comparación con los pacientes sin bronquiectasias (p = 0.002, 0.005 y 0.014 respectivamente). Así mismo, presentaron una mayor frecuencia de exacerbaciones de asma (p <0.001) y un peor control de la enfermedad (ACT 21 vs 16pts, p <0.001). Los pacientes con asma y bronquiectasias también presentaban más sinusitis (10.3% vs 2.1%, p = 0.018) y pólipos nasales (28.2% vs 13.0%, p = 0.005), pero menos asma atópica (43.6% vs 63.0%, p = 0.005) que aquellos sin bronquiectasias. Los factores asociados independientemente con la presencia de bronquiectasias fueron la edad avanzada (42-65 años: OR, 3.99; IC 95%, 1.60 a 9.95, P = 0.003; ≥ 65 años: OR, 2.91; IC 95%, 1.06 a 8.04, P = 0.039), la gravedad del asma (OR, 8.91; IC 95%, 3.69 a 21.49; P <0.001) y las exacerbaciones (OR, 4.43; IC 95%, 1.78 a 11.05; P <0.001). En pacientes con asma grave, la edad de inicio del asma (OR, 1.02; IC del 95%, 1.01 a 1.04; P = 0.015) y las exacerbaciones (OR, 4.88; IC del 95%, 1.98 a 12.03; P = 0.001) se asociaron independientemente con el desarrollo de bronquiectasias.

Con respecto al fenotipo inflamatorio, la inflamación neutrofílica fue el fenotipo principal en ambos grupos de pacientes con asma grave. Casi el 80% de los pacientes presentaron inflamación granulocítica (eosinofílica, neutrofílica o mixta). Se observaron niveles más altos de TGFβ1, VEGF e IFNγ en los pacientes con asma y bronquiectasias (AB) en comparación con los controles (AC) (15 vs 24 pg / ml, p = 0.014; 183 vs 272 pg / ml, p = 0.048; 0.85 vs 19 pg / ml, p <0,001 respectivamente). El factor estimulante de colonias de granulocitos y macrófagos (GM-CSF) disminuyó significativamente en el grupo AB (1.2 vs. 4.4 pg / ml, p <0.001). Los niveles de IL-8, elastasa neutrófilica y TNFα no presentaron diferencias significativas entre ambos grupos.

En conclusión, la prevalencia de bronquiectasias es alta en pacientes con asma, especialmente en aquellos con asma grave. En estos pacientes, la edad de aparición del asma y las exacerbaciones fueron factores independientes asociados con la aparición de bronquiectasias. El tipo de inflamación en pacientes con asma no difirió según la presencia o ausencia de bronquiectasias. Se observó un incremento de biomarcadores

de remodelado de las vías respiratorias como TGFβ1 y VEGF en pacientes con asma y bronquiectasias.

PALABRAS CLAVE: asma; bronquiectasias; inflamación; citoquinas

INTRODUCTION

1. Introduction

1.1. Asthma

1.1.1. Definition and epidemiology

According to the GINA 2019, asthma is a heterogeneous disease characterized by chronic airway inflammation. It is defined by two key features: a history of respiratory symptoms such as wheezing, shortness of breath, chest tightness and cough that vary over time and in intensity, plus a variable expiratory airflow limitation. This airflow limitation may become persistent later in the course of disease. (1)

Asthma is the world's most prevalent chronic respiratory disease, between 1990 and 2015 its prevalence rose by 12.6%. (2) Currently, asthma affects 339 million people worldwide. (3) Around 1000 people die every day and the prevalence of asthma continues to increase, especially in low and middle-income countries, where the most severe cases are recorded. (3) In Europe, asthma affects 5-10% of the adult population (in Spain, 5%). Similarly, the prevalence of asthma in China is 4.2%. (4) It is estimated that by 2050, asthma will affect approximately one billion people worldwide; so it remains a great burden and challenge to patients, to their families, and to the healthcare systems. (5,6) Asthma is more likely to affect the female population: in adults over 35 years of age, it is 20% more frequent in women than in men. However, in children, the prevalence is just the reverse. The gender difference in the prevalence of asthma is a complex entity which may be partly explained by hormone-gene interactions or by factors related to lung innate and acquired immunity development. (7)

1.1.2. Asthma phenotypes

Asthma is increasingly being recognized as an umbrella term covering various asthma subgroups, known as phenotypes or endotypes. (8) Classically asthma was considered as a disease associated with atopy and/or allergy, which begins in childhood and may or not persist into adulthood. (9) Today, however, asthma is recognized as a heterogeneous and multifactorial disease including different phenotypes, each one with its own natural history and a different response to treatment, based on clinical or physiological characteristics, radiological patterns, triggers factors or type of inflammation in sputum and blood. (10–12) The identification of asthma subtypes is critical for the personalized treatment of asthma on the basis of understanding underlying genetic bases, molecular mechanisms, treatment response and prognosis. (13) However, the overlap between different asthma

entities makes it difficult to recognize a particular phenotype and to apply specific treatment strategies accordingly. Haldar et al (14) used a multivariate mathematical technique (k-means cluster analysis) to detect distinct asthma phenotypes, and demonstrated differences in clinical response to treatment algorithms. The study identified three clusters: cluster 1, early-onset atopic asthma, accompanied by evidence of airway dysfunction, symptoms, and eosinophilic airway inflammation; cluster 2, predominately female obese subjects characterized by evidence of asthma symptoms, but without eosinophilic airway inflammation; cluster 3, benign asthma, in which 58% of patients had no evidence of significant airway hyperresponsiveness. This study shared some common features with the cluster analysis led by the Severe Asthma Research Program (SARP), sponsored by National Heart Lung and Blood Institute. (15) Recently, a new study (16) based on proteomic analysis of sputum supernatants classified 10 subgroups of asthma at molecular level. In summary, this classification was finally categorized into three main clusters: eosinophilic, neutrophilic and atopic with low granulocyte inflammation.

A summary of the different asthma phenotypes is shown in table 1.1.

Table 1.1. Summary of asthma phenotypes and endotypes

Inflammatory features (sputum)	Annotation
Eosinophilic	Sputum eosinophils ≥ 3%
Neutrophilic	Sputum neutrophils ≥ 65%
Mixed	Both eosinophils ≥3% and neutrophils ≥ 65%
Paucigranulocytic (17)	Neither eosinophils ≥3% nor neutrophils ≥65%
Clinical features	Annotation
Childhood asthma	Most children with asthma present symptoms before the age of five. Childhood asthma is also associated with atopy (95%)
Adult-onset asthma	More severe and more difficult to control. Patients often require higher doses of ICS. Occupational asthma should be ruled out.
Obesity-associated asthma (18)	BMI >30kg/m^2. Prominent respiratory symptoms but little eosinophilic airway inflammation. Serum IL-6 may be a marker of asthma severity in obese patients.
Exacerbation-prone asthma (19,20)	At least one exacerbation in the previous year

Cough variant asthma (21)	Atypical asthma in which cough is the only symptom: sensitive to cough suppression therapy.
Treatment	Annotation
Severe refractory asthma	Found in 5-10% of the adult population, with older age, longer duration of disease, lower lung function (SARP cohort) (15)
Corticoid-dependent	Need for daily administration of oral corticosteroids; usually more eosinophilic and with a high IgE level.
By triggering factor	Annotation
Aspirin-induced asthma (AIA) (22)	ASA triad; Patients usually have asthma, nasal congestion and recurrent nasal polyps.
Exercise-induced asthma	The key point is to take a preventive albuterol inhaler about 15 to 20 minutes before exercise.
Cold/dry weather	Not well known
Perimenstrual asthma (PMA) (23)	Not well known. A strong association between PMA and aspirin-sensitive asthma has been found.
Environmental allergens	HDM, pollen, animal dandruff, cockroach, etc.
Atopy	Annotation
Allergic (high Th2, extrinsic)	Skin prick test (+) or high level of serum IgE level
Non-allergic (low Th2, intrinsic)	Absence of IgE antibodies to environmental allergens
Work-related asthma (WRA) (24,25)	Annotation
Occupational asthma	Persulfate salts; asbestos; nickel sulfate; animal protein, etc.
Work-exacerbated asthma	Preexisting asthma that can be worsened by workplace conditions.
Other Syndromes	Eosinophilic bronchitis, asthma-like disorders
Radiological findings	Annotation
Airway dilatation	Bronchiectasis
Bronchial wall thickening	Causes accumulation of mucus

Air trapping	Indirect evidence of small airway disease, correlated with asthma severity
Small airway asthma (26)	Suboptimal asthma control (ACQ>1.5pts) with small airway dysfunction

1.1.3. The pathophysiology of asthma

A large proportion of current research in asthma is aimed at identifying the immunological pathways that determine the disease, including disease phenotypes and endotypes and certain biomarkers that may help us design precision medicine approaches. (27,28) It is generally agreed that three possible immune responses can be distinguished in asthma development, namely type 2 immune response, non-type 2 immune response, and mixed type Th2/Th17 immune response, (29) 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 at any grade of severity, although in mild asthma it is also associated with early onset and atopy. (30,31) The distinctive feature of this response is that the Th2 pathway 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. (32) IL-5, along with GM-CSF, play essential roles in the activation and survival of eosinophils. IL4 and IL-13 are required for airway responsiveness and mucous metaplasia. 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. (33) Before differentiation, both pathways appear to have common bronchial epithelium-derived alarmin cytokines, such as IL-33, IL-31, IL-25 and thymic stromal lymphopoietin (TSLP), and these molecules are currently under investigation as possible therapeutic targets. (Figure 1.1) (34) 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.

The non-type 2 immune response, also known as non-eosinophilic asthma (NEA), accounts for about 50% of severe asthma cases. (35) The most relevant clinical trait of NEA is its poor response to standard asthma treatments, especially to inhaled corticosteroids, resulting in greater disease severity and difficult-to-control asthma. (35) NEA appears to include patients with Th17-dependent neutrophilic inflammation, patients with neutrophilic inflammation dependent on the dysregulation of the innate immune response associated with IL-1b or CXCR2, (36) and patients with neurogenic inflammation

basically associated with the RTPA1 receptors. (37,38) These response types have been the focus of several studies and the putative role of tumour necrosis factor-alpha, IL-6, IL-8, IL-37, IL-22 and IL-9 is under debate. (37) Moreover, neutrophilic inflammation is also observed in patients with work-related asthma. (35) Additionally NEA encompasses the other group of asthma patients who present no evidence of increased numbers of eosinophils or neutrophils in sputum or blood, known as paucigranulocytic asthma (PGA). Although underestimated, this seems to be the most common asthma subtype in patients in stable condition. (17)

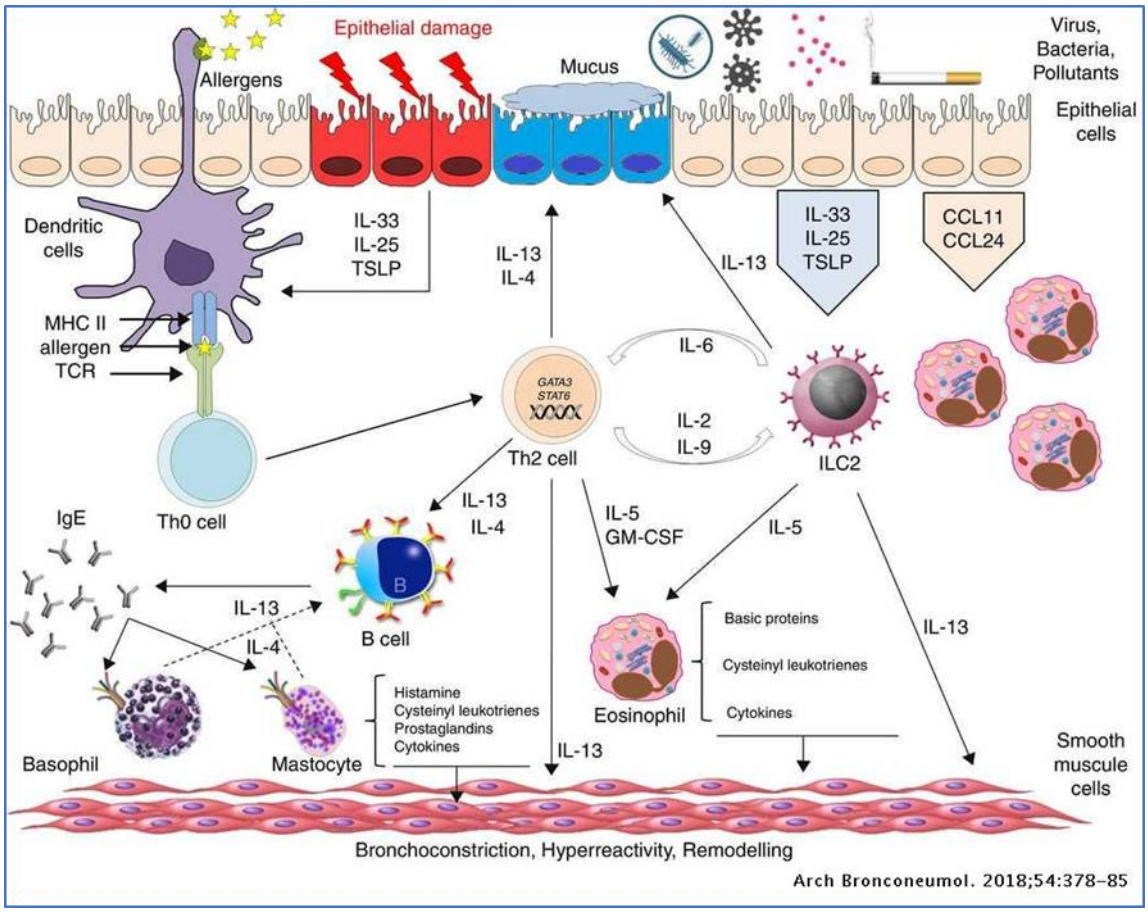

Figure 1.1. Main immunological pathways involved in type 2 response in bronchial asthma. Muñoz X et al. (34)

Although patients with a mixed Th17/type 2 response have been reported, the relationship between the two response pathways is highly complex and little understood. In an atopic dermatitis animal model, 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. (39) At the same time, in murine schistosomiasis models IL-4 and IL-13 seem to be capable of amplifying the Th17 response by regulating CD209a

expression in dendritic cells. (40) 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. (41) The best known asthma phenotypes today and their inflammatory pathways are summarized in figure 1.2.

Airway remodelling is another fundamental change that occurs in asthma patients. The main changes include hyperplasia of epithelial goblet cells, hypertrophy of bronchial smooth muscle, transformation of fibroblasts to myofibroblasts, deposition of subepithelial collagen, thickening of the lamina reticularis, and even proliferation of airway blood vessels and nerves. (42) Early cognition suggests that airway changes are the consequence of the persistent inflammation; however, the inflammation by itself cannot account for the emerging of tissue restructuring in young children who present clinical symptoms of asthma some years later. The airway inflammation and remodelling together likely explain the clinical manifestations of asthma. (43,44)

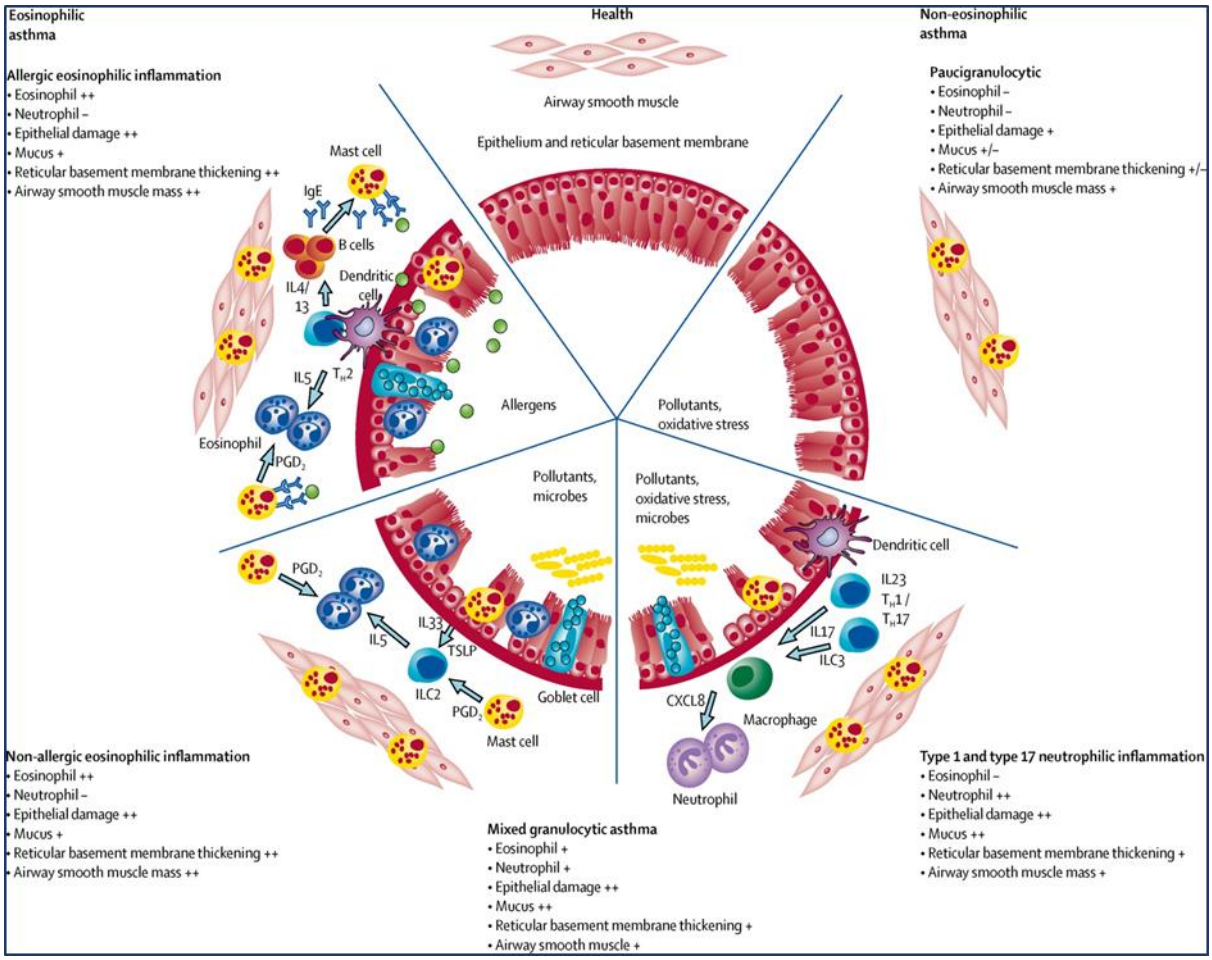

Figure 1.2. The pathogenesis of different phenotypes of asthma. Papi et al (45)

1.1.4. Severe asthma

The ERS/ATS guideline (46) defines severe asthma as asthma that requires treatment with high dose inhaled corticosteroids (ICS) plus a second controller and/or systemic corticosteroids to prevent it from becoming “uncontrolled”, or which remains “uncontrolled” despite this therapy. According to the GINA guideline, the term “severe asthma” refers only to those patients after eliminating contributory factors. (1) However, due to the heterogeneity in the pathophysiology and gene-environmental interaction, the asthma phenotype is also difficult to determine. The same occurs in severe asthma, as there may be overlapping between phenotypes. Severe asthma is also characterized by accompanying comorbidities; according to an Italian severe asthma registry study, (47) approximately 70% of subjects had allergic rhinitis, and 42.6% had chronic rhinosinusitis with nasal polyps (CRSwNP). Data from the International Severe Asthma Registry yielded similar results but reflected a strict treatment strategy where 51.1% of patients were receiving regular intermittent oral corticosteroids and 25.4% were receiving biologics. (48) Using predominantly clinical characteristics the Severe Asthma Research Program (SARP)identified five clusters of distinct clinical phenotypes of asthma: cluster 1: childhood-onset/atopic asthma characterized by younger, predominantly female subjects with normal lung function; cluster 2: primarily childhood-onset/atopic asthma in slightly older subjects, two-thirds female with a baseline prebronchodilator lung function that is either relatively normal (65% with a predicted FEV1>80%) or can be reversed to normal in nearly all subjects (94%); cluster 3: late-onset/non-atopic asthma in older female subjects with high body mass index (BMI, mean 33 kg/m²), a decreased baseline pulmonary function (71% with predicted FEV1<80%), and requirement of complicated medical regimens; clusters 4 and 5: childhood-onset (72%) and late-onset (69%) respectively, both characterized by a long duration of disease, but differing in the level of baseline lung function and the magnitude of response to bronchodilators. Patients in cluster 4 tended to reverse to near-normal lung function after bronchodilator treatment whereas those in cluster 5 did not (fixed obstructive defect). Nearly 70% of subjects in cluster 4 and 80% of subjects in cluster 5 met the ERS/ATS workshop criteria for severe asthma. (15)

Recently, Lefaudeux and colleagues (49) performed a cluster analysis based on sputum proteomics and transcriptomics data in patients with moderate-to-severe asthma in whom four stable phenotypes of asthma were identified. Eight pre-specific clinical and physiological parameters were used in this cluster including age of onset, tobacco packs-year, lung function, exacerbation during the previous year, ACQ and dose of oral corticosteroids. Cluster 1 comprised well-controlled moderate-to-severe asthmatics, while

clusters 2, 3 and 4 were composed of severe asthma patients. Cluster 2 were characterized by late-onset severe asthmatics with a history of smoking and chronic airflow obstruction. Cluster 3 was formed by non-smoking severe asthma patients with chronic airflow obstruction. Cluster 4 comprised mainly obese female patients with uncontrolled severe asthma and increased exacerbations, but with normal lung function. The study always suggested a higher level of eosinophils in the severe asthma clusters when comparing cluster 1 with moderate-severe asthma. However, no differences were found in sputum neutrophils counts, exhaled nitric oxide or serum IgE levels. (Figure 1.3)

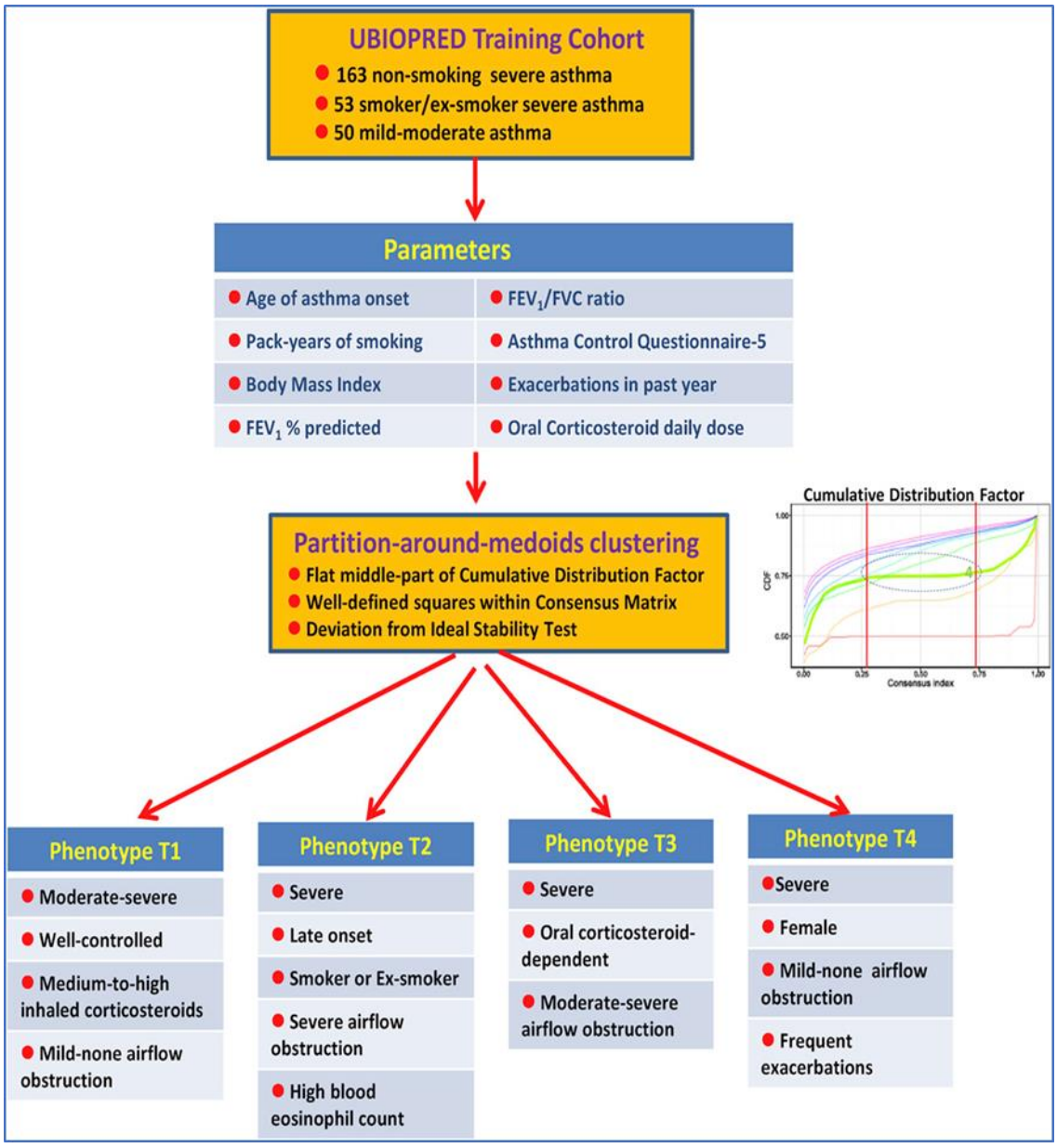

Figure 1.3. Clinical phenotypes of moderate-severe asthma derived from the U-BIOPRED cohort clustering analysis based on eight clinical-physiologic parameters. (49)

1.1.5. Diagnosis

The diagnosis of asthma is based mainly on clinical symptoms together with airway obstruction and reversible airflow restriction. A detailed anamnesis is essential for the diagnosis of asthma, with questions on personal and family respiratory disease history, allergy, time of onset of symptoms and mitigating factors. Wheezing is a key sign for the initial consideration of asthma diagnosis after adjusting for other obstructive respiratory diseases. If the clinical history is not enough to base a diagnosis decision, complementary test procedures should be performed to confirm the diagnosis. Figure 1.4 shows a Spanish asthma diagnostic algorithm.

List of objective tests:

- Spirometry: includes the basic forced spirometry and a bronchodilator test. Spirometry is an essential tool for an initial evaluation of lung function and for assessing whether the patient presents an obstructive or restrictive pattern.
- Peak expiratory flow (PEF): an objective measure of airflow limitation, predominantly assessing the variability of large airway calibre. Variability of maximum diurnal peak expiratory flow above 20% for more than two weeks is of clinical interest.
- Airway responsiveness: Assessed by bronchial provocation tests: direct (e.g., methacholine or histamine) or indirect (e.g., exercise, allergen, mannitol).
- Fractional exhaled nitric oxide (FeNO): a noninvasive biomarker in asthma. In adults, FeNO below 25 ppb implies the absence of eosinophilic airway inflammation; FeNO above 50 ppb suggests eosinophilic airway inflammation; FeNO between 25 and 50 ppb is a suspicious range in which reference to the clinical situation is needed.
- Induced sputum cell differential count: a noninvasive biomarker in asthma. It provides a reliable basis for asthma phenotyping and can monitor steroid responsiveness in asthma treatment.
- Specific inhalation challenge: for patients with suspected occupational asthma.
- Skin prick testing (SPT) and specific IgE determination: used to determine specific allergens.

The diagnosis of patients with severe asthma is sometimes difficult due to the complexity and natural process of the disease. Many diseases may mask severe asthma. Therefore, for patients with severe asthma, in addition to the evaluation of their clinical history further studies like chest high-resolution CT and bronchoscopy are also required. Some of these

pathologies that may mimic asthma symptoms should be ruled out previously, such as bronchiolitis obliterans, bronchiectasis/cystic fibrosis, hypersensitivity pneumonitis, hypereosinophilic syndromes, allergic bronchopulmonary aspergillosis (ABPA), acquired tracheobronchomalacia, Churg-Strauss syndrome, etc. (50)

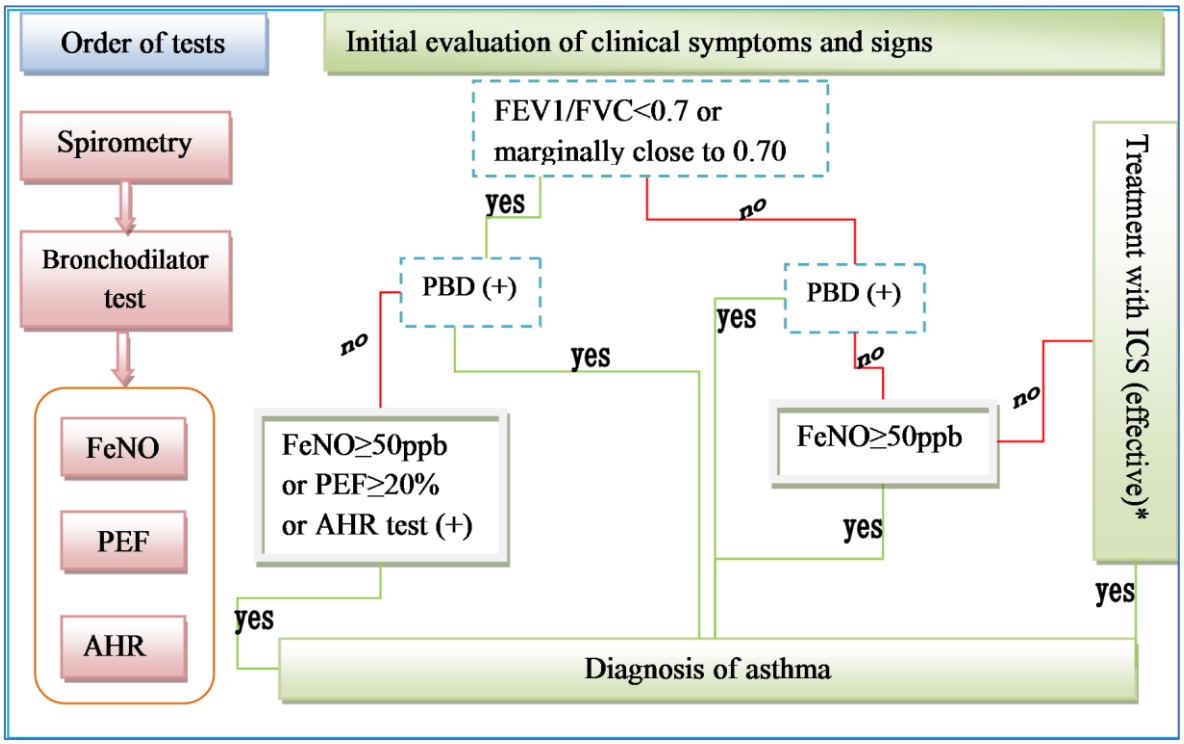

Figure 1.4. Asthma diagnostic algorithms. Adapted from the GEMA 4.3 (5)
PBD (+): Increase in FEV1 of >12% and >200 ml from baseline, 10–15 minutes after 200–400 mcg salbutamol or equivalent. PEF (+): Variability ⩾20% more than 3 days weekly in adults. *An increase in FEV1 by >12% and >200 ml (or PEF by >20%) from baseline after 4 weeks of treatment, outside any respiratory infections. AHR test: Airway hyperresponsiveness (methacholine or histamine)

1.1.6. Treatment

There are no definitive therapies for asthma, but it can be managed with proper prevention of attacks and appropriate treatment. The main objective of asthma management is the control of the disease, by minimizing long-term symptoms, preserving physical activity, preventing the risk of exacerbations, avoiding deterioration of lung function and finally reducing long-term mortality. The treatment of asthma depends on a stepwise approach based on inflammatory severity. Various scientific societies recommend different ways to escalate treatment, the most used in our environment being the GEMA (5) and GINA (51) guidelines. Altering a strategy that had been in place for

more than 35 years, the GINA guidelines recently proposed initiating treatment on demand with a combination of inhaled corticosteroids plus formoterol for asthma scales 1 and 2 (intermittent and mild asthma) (Figure 1.5). (45) However, this recommendation is questioned by both the FDA and the EMA, which have not approved this combination for the treatment of asthma at these therapeutic stages.

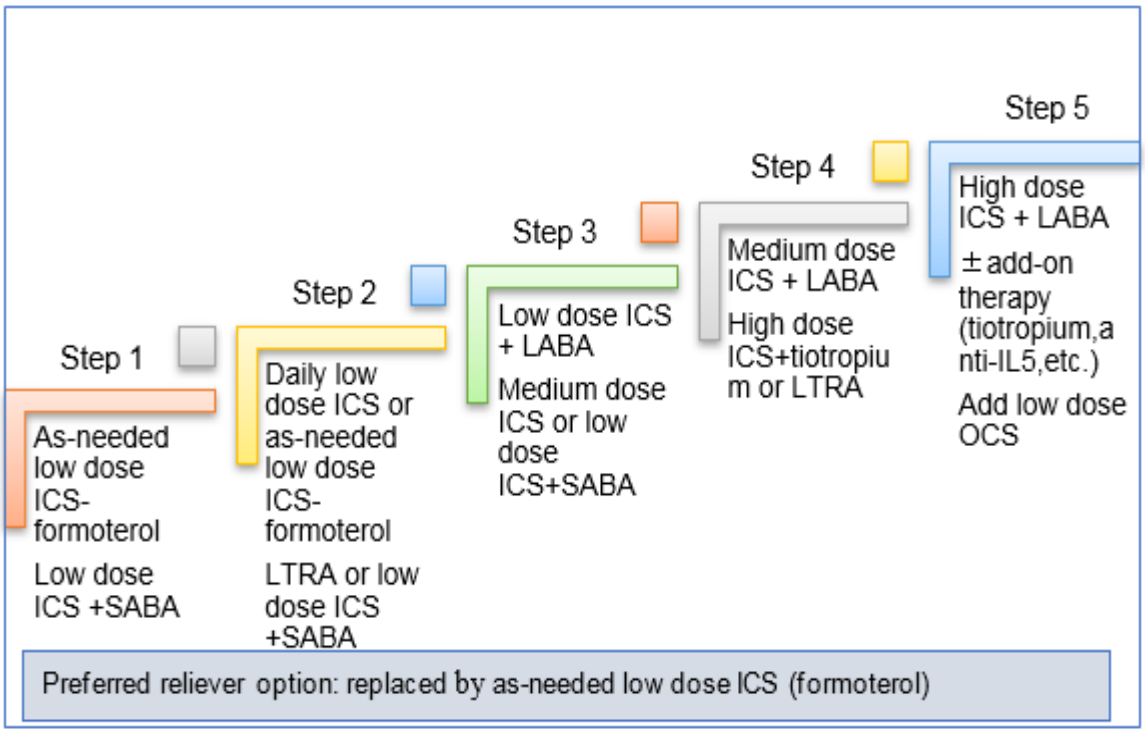

Figure 1.5. Based on the 2019 Global Initiative for Asthma (GINA) treatment strategy for adults and adolescents. ICS: inhaled corticosteroids; SABA: short-acting β2-agonists; LTRA: leukotriene receptor antagonists; LABA: long-acting β2-agonists; OCS: oral corticosteroids.

The management of asthma is a continuous, dynamic process. This cyclical adjustment of treatment implies that it should be evaluated objectively and periodically to maintain control. Step-up and step-down therapies may be administered. Three step-up strategies have been suggested: step-up long-term (SLT), step-up short-term (SST) and step-up intermittent (SUI) therapies. SLT is indicated for patients who have been uncontrolled over a long time and who require an increase in overall medication. SST occurs during a temporary loss of acceptable control, for example at the onset of a viral respiratory tract illness which requires an increase in baseline ICS. SUI refers to the intermittent use of ICS/LABA or ICS/SABA combinations for the day-to-day treatment of variable asthma symptoms. (52) A step-down medication is recommended when the patient is satisfactorily controlled for more than three months.

No pharmacological treatments Asthma is caused by a combination of internal and environmental effects. These interactions may occur early in life, even in the fetal phase during pregnancy. A variety of environmental factors, including biological and social factors, may play an important role in the development of asthma where risk factors are concentrated in nutrition, allergens, environmental pollution, microbes, and psychosocial factors. A preventive strategy focused on avoiding exposure to these risk factors may achieve comprehensive asthma control and prevent asthma exacerbations.

Educational intervention is the other fundamental principle for clinical practitioners. The purpose of education is to improve patient compliance and to guide the asthma action plan. It should include instruction on inhaler techniques and on the habit of PEF record-keeping, with a detailed written diary provided that allows patients to adjust their therapy within the recommendations. Poor compliance and inappropriate use of inhaled drugs are factors that increase the difficulty of asthma control, (53) while proper use of inhalation devices, good compliance, and asthma education are all likely to improve it.

Pharmacological treatments Drugs used for the management of asthma are classified into two categories: for control or maintenance, or for acute exacerbation or rescue.

Maintenance medication: Inhaled glucocorticoids (ICS), leukotriene receptor antagonist, beta 2 long-acting adrenergic agonist, tiotropium, azithromycin and monoclonal antibodies including anti-IgE, anti-IL4, anti-IL5 when necessary.

Rescue medication: as-needed low dose ICS + formoterol is prioritized as reliever option in the latest GINA recommendations, or a β_2-agonist in the GEMA guidelines.

SABA inhalers have been the first-line treatment for asthma for 50 years. However, the new recommendation of GINA is that regular long-term treatment with SABA alone is harmful, raising the occurrence of adverse effects including increased allergic response, β-receptor down-regulation and decreased bronchi-protection. (54) Reddel (55) et al suggested that ICS should not be restricted to patients with symptoms more than two days per week, but should be extended to those with mild asthma in order to reduce the risk of exacerbation and prevent subsequent symptoms. A Cochrane review suggested that vitamin D had a beneficial effect on asthma attacks and emergency room visits in patients treated with systemic corticosteroids. (56)

Allergen-specific immunotherapy (AIT) is another potential treatment approach in allergic diseases. The subcutaneous injection of common allergens (such as dust mites, ragweed, etc.) can reduce symptoms of asthma and airway hyper-responsiveness. (57)

Asthma control and treatment monitoring

For the monitoring of asthma control, the easiest and most accessible approach in clinical practice is the administration of an Asthma Control Test (ACT) (58) or an Asthma Control Questionnaire (ACQ). (59) ACQ scores consistently above 1.5 or ACT scores below 20 indicate uncontrolled asthma. The NICE guideline provides the following criteria for the monitoring of asthma: lung function test, measurement of FeNO, peripheral blood eosinophil count, airway responsiveness determined by challenge tests (indirect or direct), adherence to treatment, inhaler technique and telehealth care. (60)

The monitoring strategies can be categorized into three levels: self-monitoring, physician intervention, and health care management. The self-monitoring of asthma consists of a written action plan and a regular medication review by a physician. Patients can monitor both asthma symptoms and PEF-based lung function. Whether PEF monitoring should be long or short-term during the ongoing management of asthma remains a controversial issue. Randomized trials have not shown any improvement in asthma outcomes with PEF monitoring over symptom-based diaries. (61) The GINA guidelines recommend the use of PEF especially for assessments of asthma control in patients with moderate-to-severe asthma. (62) The amount of diurnal variation is usually less than 20% when asthma is well-controlled; however, an evidence-based systematic review showed that good asthma self-management can reduce the relative risk of hospitalization for asthma by 39%. (63) Physicians play a key role in the assessment of asthma by identifying trigger factors, investigating the underlying mechanism, detecting complications and applying adequate treatment. Other health professionals also play their part in the monitoring of asthma control, including pharmacists, physiotherapists, and nursing staff.

Precise and individualized treatment for asthma

As the specific characteristics of each phenotype with its different underlying inflammatory mechanisms may result in different responses to treatment, a new prospective approach for precision medicine should be adopted. (64) In asthma subjects with either allergic or eosinophilic backgrounds, monoclonal treatment blocking the effects of IgE (omalizumab), IL5 (mepolizumab, reslizumab), IL5Rα (benralizumab) and IL4 Rα (dupilumab) and targeting the interleukin pathway has led to the formulation of a range of antibody

therapies. (65,66). Other targeted biological therapies have been developed to blockade the innate cytokines IL-25, IL-33 and TSLP. (67) (Figure 1.6)

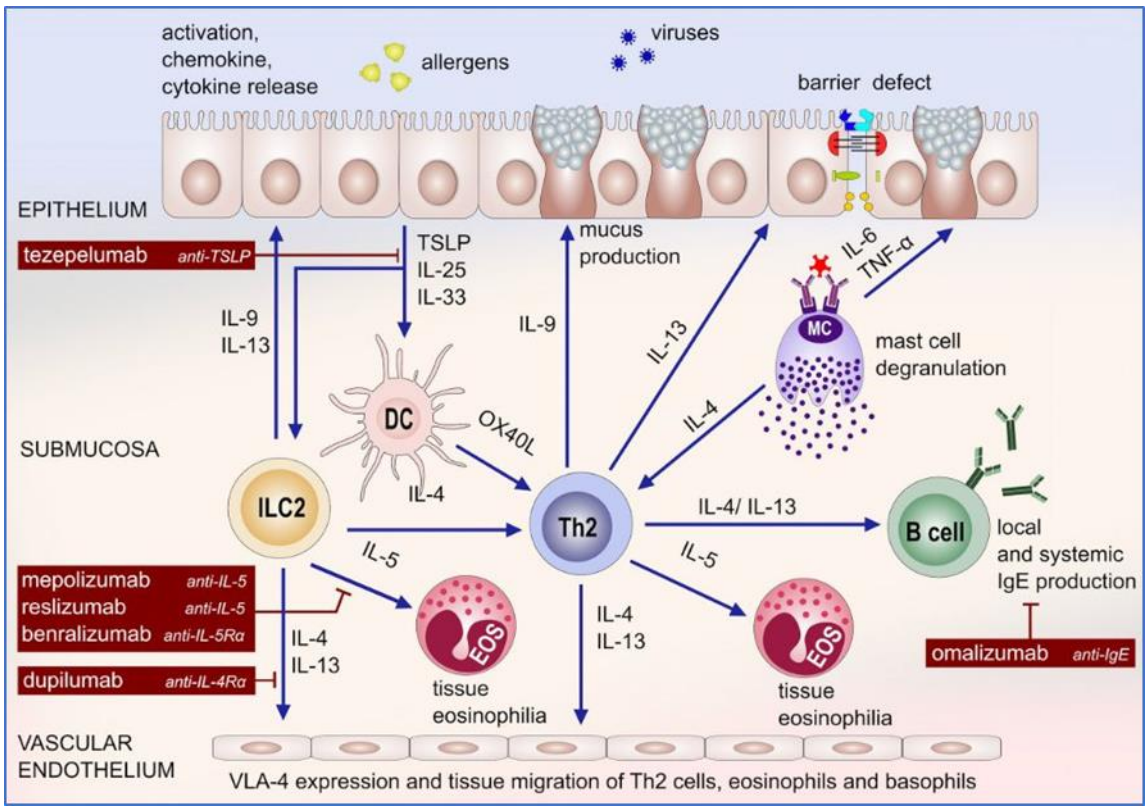

Figure 1.6. Targeted biological therapies of T2 asthma. Seys et al. (67).

Eos, eosinophil; ILC2, innate lymphoid cell type 2; MC, mast cell; Th2, T helper 2 cell; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin; VLA-4, very late antigen-4.

1.2. Bronchiectasis in asthma patients

1.2.1. Definition of bronchiectasis

At present, the definition of bronchiectasis is based on radiological features, which reveal a relatively increased and irreversible bronchial dilatation in comparison with the adjacent artery. Clinically the bronchiectasis is expressed by chronic productive cough, purulent expectoration, breathlessness (wheeze), hemoptysis, and chest pains, and it is usually associated with recurrent chest infections. (68) A persistent or repeated airway inflammation can lead to a progressive decline in pulmonary function and a deterioration in quality of life. (69) According to the form of bronchial dilatation, bronchiectasis can be classified into three types radiologically: 1) cylindrical, characterized by a uniform dilation of the bronchi; 2) varicose, in which the bronchi are dilated with multiple indentations; and 3) cystic, which ends in large cysts or saccules. Cylindrical bronchiectasis accounts for about 85% of all cases. MPPs such as Hemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumonia, Moraxella catarrhalis and Staphylococcus aureus are the microorganisms most frequently involved in the pathogenesis of bronchiectasis. (70)

For many years, chest radiography has been used for initial screening of airway abnormality in which certain characteristic signs have been found such as tram track, ring sign, and branching opacities. In 1985 Jacobsen et al (71) designed the first study to compare the efficiency of Rx and CT scan in the diagnosis of bronchiectasis, which raised the sensitivity of bronchiectasis diagnosis from 50% to 90%. Since then, the CT scan has been widely used instead of simple radiography and bronchography. Currently, high-resolution computed tomography (HRCT) is the gold standard for diagnosing bronchiectasis. With the continuous updating of CT technology, a multidimensional score scale has been developed for digital evaluation of the bronchiectasis severity and even for predicting the risk of exacerbation. (72–76) (Figure 1.7)

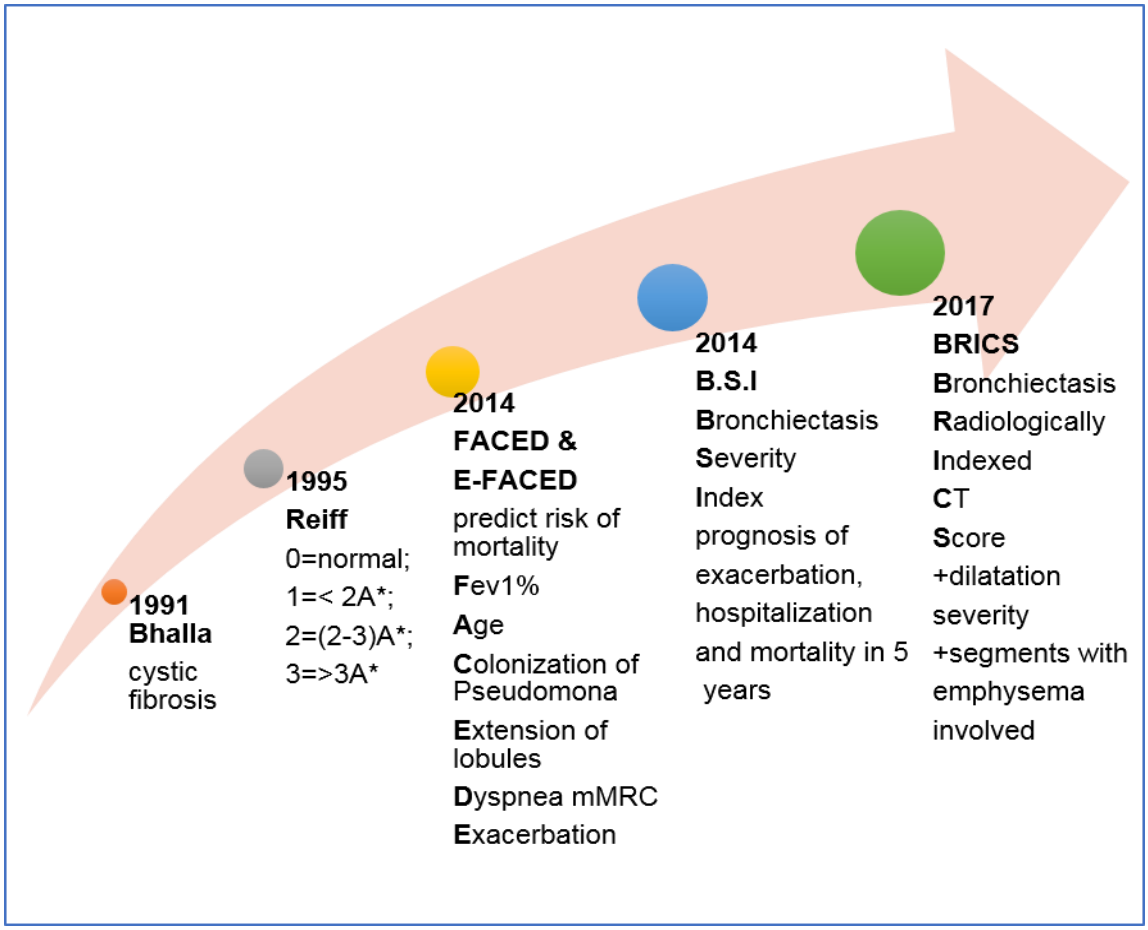

Figure 1.7. A historical review of different scoring scales on the evaluation of bronchiectasis. * Diameter of bronchial lumen in comparison with adjacent artery.

1.2.2. Prevalence of bronchiectasis in asthma

Although asthma and bronchiectasis are two different disease, they often coexist, especially in patients with severe asthma. Today bronchiectasis is the third most common chronic inflammatory disease of the airway after asthma and chronic obstructive pulmonary disease (COPD), and it is quite frequent to see an overlap of asthma-bronchiectasis and asthma-COPD. (77) The prevalence of bronchiectasis has been estimated at 53 to 566 cases per 100,000 inhabitants, with the elderly and female population being more likely to suffer more from the disease. In fact, the prevalence of bronchiectasis may still be underestimated, in part because of the large number of bronchiectasis patients with a primary diagnosis of COPD or asthma. (78,79) Although the presence of bronchiectasis in asthma patients is not universal, its prevalence seems to be a relevant clinical issue; according to different studies with a variable population recruitment criterion, its prevalence ranges between 3% and 80% in patients diagnosed with asthma. (Table 1.2).

Table 1.2. Prevalence of bronchiectasis in asthma patients.

Year	1st author	Prevalence	n	Design and asthma severity
2007	Oguzulgen I. (80)	3%	1680	Retrospective; General population
2013	Lujan M (81)	12.0%	100	Prospective; SDA 20% vs. NSDA 4% *
1997	Park JW. (82)	17.5%	57	Retrospective; Without specification of asthma severity
2018	A. Padilla-Galo (83)	28.4%	398	Prospective; Uncontrolled mod-severe asthma
2012	Khadadah M. (84)	28.6%	28	Retrospective; Mild and severe asthma (42.8%), Moderate (57.1%)
2019	Clemente MG (85)	35.0%	108	Retrospective; Severe asthma
2018	Kim S. (86)	35.2%	91	Retrospective; Severe asthma;
2011	Menzies D. (87)	35.3%	133	Cross-sectional; Severe asthma
2009	Gupta S. (88)	40.0%	463	Cross-sectional; Severe asthma
2018	Coman I. (89)	47%	184	Retrospective; Severe asthma
2017	Dimakou K. (90)	67.5%	40	Prospective; Severe asthma
1996	Paganin F. (91)	20-80%	126	Retrospective; According to allergic status and AAS asthma severity score

* SDA: severe steroid-dependent asthma, NSDA: non-steroid dependent asthma.

1.2.3. Role of asthma in the pathogenesis of bronchiectasis

Asthma patients with bronchiectasis usually have a higher risk of exacerbation and hospitalization. (80) However, whether asthma directly leads to the development of bronchiectasis is still a controversial issue. In the study led by Lonni et al (92), the etiology was identified in 60% of all enrolled patients: post-infective (20%), COPD-related (15%), connective tissue disease-related (10%), immunodeficiency-related (5.8%), and asthma-related (3.3%). The British guideline suggested that asthma should be considered as a cause of bronchiectasis if no other etiology is identified. (93) The underlying pathophysiology is not entirely clear, but the presence of bronchiectasis in asthma patients seems to be mostly associated with neutrophilic inflammation. (94)

In line with the vicious circle theory described by Cole (95) in 1986, both infection and inflammation are key components of the pathogenesis of bronchiectasis. This is because any direct damage or underlying disease of the respiratory airway impairs the first line mucociliary clearance mechanism. As a result, the accumulation of mucus will facilitate invasion and colonization of the bronchial membrane by microorganisms. (96) Certain MMPs like P. aeruginosa can release cilio-inhibitory molecules which will aggravate clearance capacity. Additionally, once the P. aeruginosa is inducted by activated neutrophils, it may produce a mutation leading to alginate production and biofilm formation that protects bacteria from phagocytosis by immune system cells like neutrophils and macrophages. (97) The main cells involved in the inflammatory process in bronchiectasis are neutrophils, lymphocytes, and macrophages. As the most prominent cell type in the bronchial lumen, neutrophils can release reactive oxygen mediators, particularly proteases/elastase which may cause bronchial dilation. In sputum, an increased concentration of elastase and proinflammatory mediators like IL-8, TNF-a and leukotriene (LT) B4 is also found. The pro-inflammatory and anti-inflammatory response leads to changes in airway structure including loss of elasticity and destruction of muscle and cartilage. (Figure 1.8)

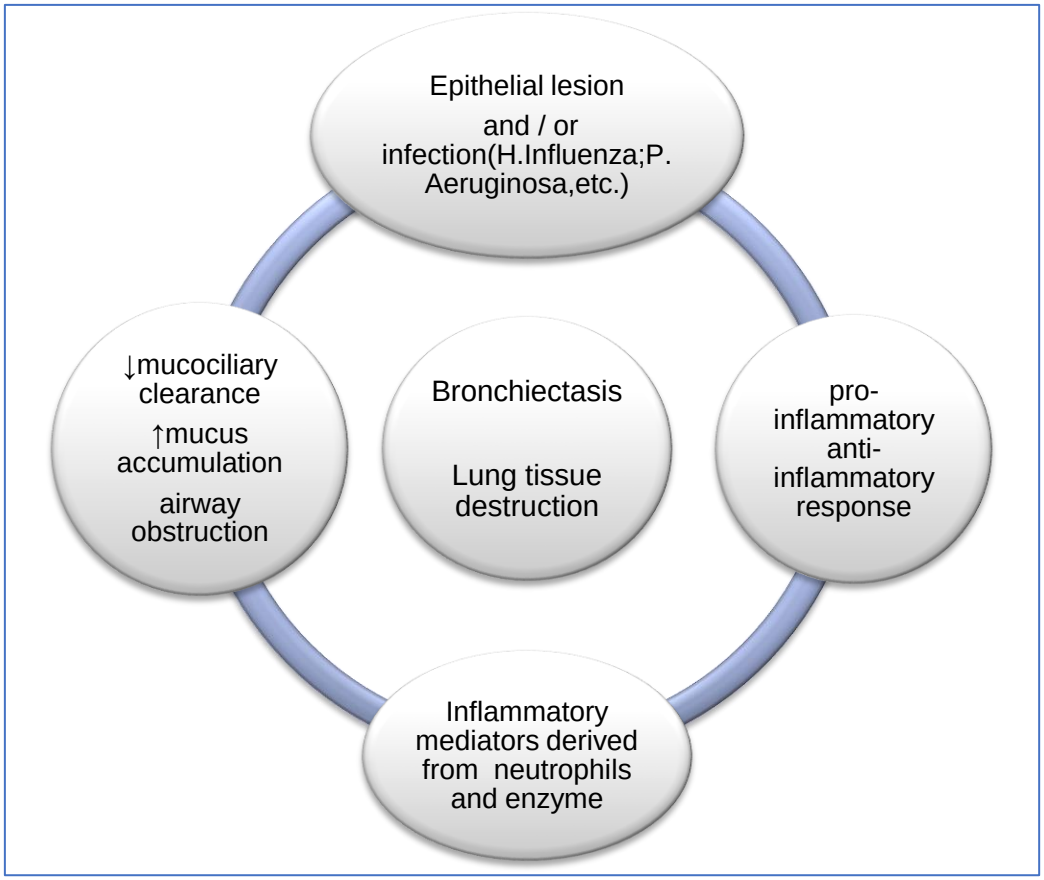

Figure 1.8. Model describing the pathogenesis of bronchiectasis.

Moreover, the imbalance between the metalloprotease (MMPs) matrix and their tissue inhibitor is considered as a potential mechanism in asthma patients which may facilitate the formation of bronchiectasis. In turn this may lead to extracellular matrix degradation, tissue destruction and subsequently to tissue remodelling. (93)

Although asthma and bronchiectasis share some common features, the underlying mechanism is not totally identical in these two airway pathologies (Figure 1.9). Regarding the mucosal inflammation, both diseases are characterized by heterogeneity and chronic inflammation. However, the predominant eosinophilic inflammation in asthma is mediated mainly by the adaptive immune response. In the case of bronchiectasis, the most commonly acknowledged theory proposes neutrophilic inflammation.

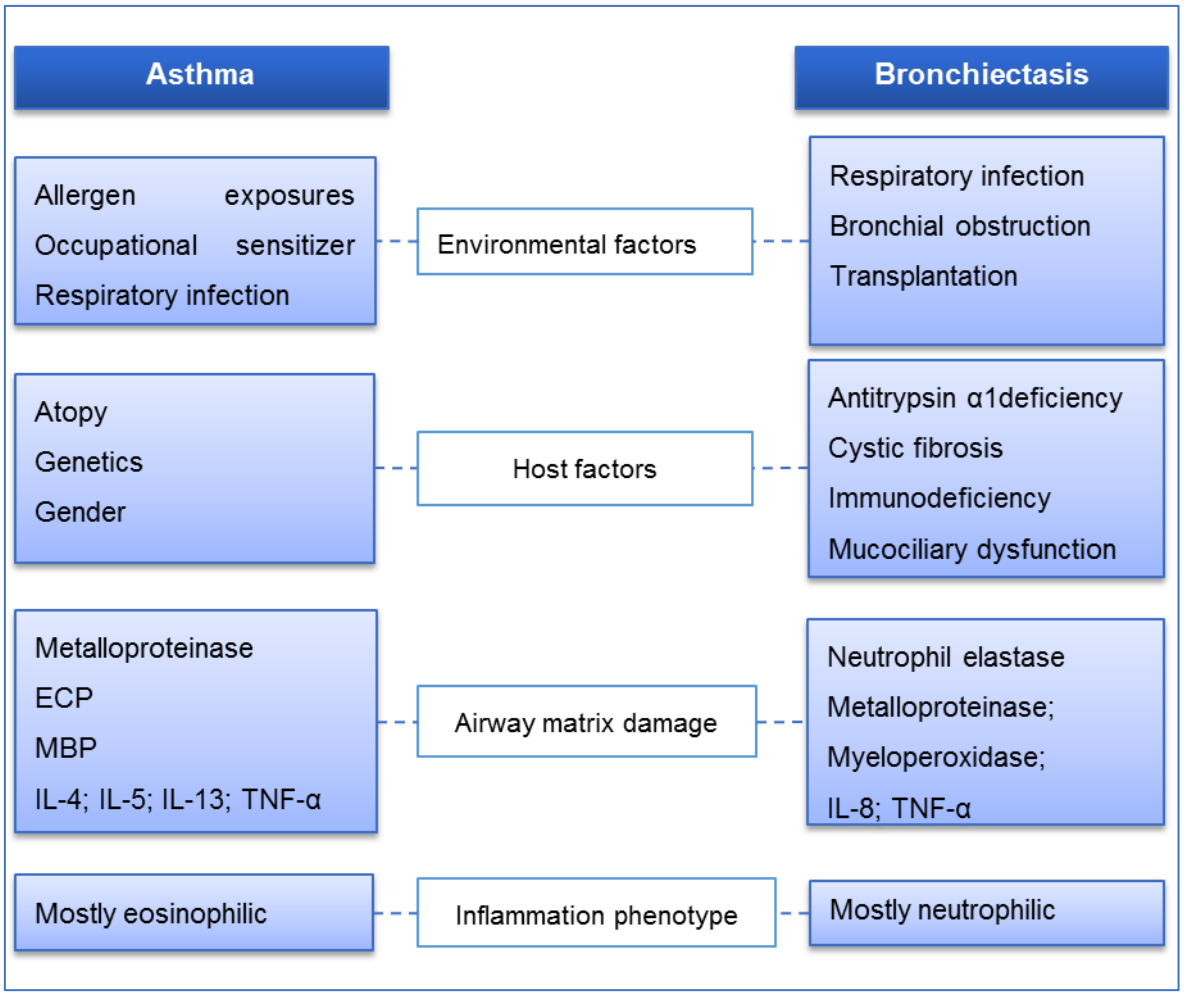

Figure 1.9. Comparison of airway inflammations in asthma and bronchiectasis. Adapted from Perez J et al. (98)

At present, concern regarding bronchiectasis is growing among clinical professionals, and the interest in studying bronchiectasis has increased significantly in the last decade. (99–102) A large international cohort study of bronchiectasis revealed that asthma as prevalent comorbidity may increase the risk of mortality. (103) However, fewer studies have estimated the impact of bronchiectasis on asthma patients; indeed, most of the published studies only pay attention to bronchiectasis without stratifying its severity and its radiological characteristics. The pathogenesis of bronchiectasis in asthma remains unclear; although bronchiectasis has been described as a new phenotype of asthma based on clinical differences, (94) this novel concept is still under debate because only limited evidence is available on the underlying mechanism. To our knowledge the inflammation profile and the differences in the role of pro-inflammatory and inflammatory cytokines in asthma patients with bronchiectasis have not been analysed. We therefore conducted two studies aiming to address the above issues.

In the first study of patients with moderate to severe asthma, the impact of bronchiectasis was estimated by comparing the basic socio-demographic data, clinical comorbidities, asthma evolution, lung function and disease management medications. Variables that presented significant differences were later included in the multivariable logistic model to identify independent factors associated with bronchiectasis. We assessed the radiological features using radiological and clinical comprehensive parameters.

In the second study, all the severe asthma patients with reliable induced sputum samples were included, and proinflammatory and inflammatory mediators representing different immunological pathways and cytokines related to airway remodelling were measured by immunoassay technique. We aimed to analyse the inflammatory phenotypes and differences in the immunological profile.

HYPOTHESES

2. HYPOTHESES

The inflammatory phenotype in asthma patients with bronchiectasis may differ from that found in patients with isolated asthma.

1. The prevalence of bronchiectasis may be underestimated especially in patients with severe asthma.

2. The presence of bronchiectasis may have a significant impact on the clinical manifestation of asthma.

3. Proinflammatory and remodelling cytokines may have different expressions in asthma patients with and without bronchiectasis.

OBJECTIVES

3. OBJECTIVES

Main objective

To study the inflammatory phenotypes of asthma patients with bronchiectasis.

Secondary objectives

1. To assess the prevalence of bronchiectasis in patients with severe asthma.

2. To quantify the expression of proinflammatory and remodelling cytokines in asthma patients with and without bronchiectasis.

3. To study the impact of bronchiectasis on the clinical manifestation of asthma.

METHODS & RESULTS

4. METHODS AND RESULTS

4.1. Study 1. Impact of bronchiectasis on moderate to severe asthma: clinical manifestation, comorbidities and disease management

Study population and design

Two hundred and sixty-nine asthma patients were seen at our specialized asthma unit in 2018. Of these, 33 with intermittent and mild asthma, and twelve with known etiology of bronchiectasis (eight Churg-Strauss syndrome, two allergic bronchopulmonary aspergillosis, and two sequelae post tuberculosis) were excluded. The remaining 224 patients with moderate and severe asthma were finally included in the study (Figure 4.1.1).

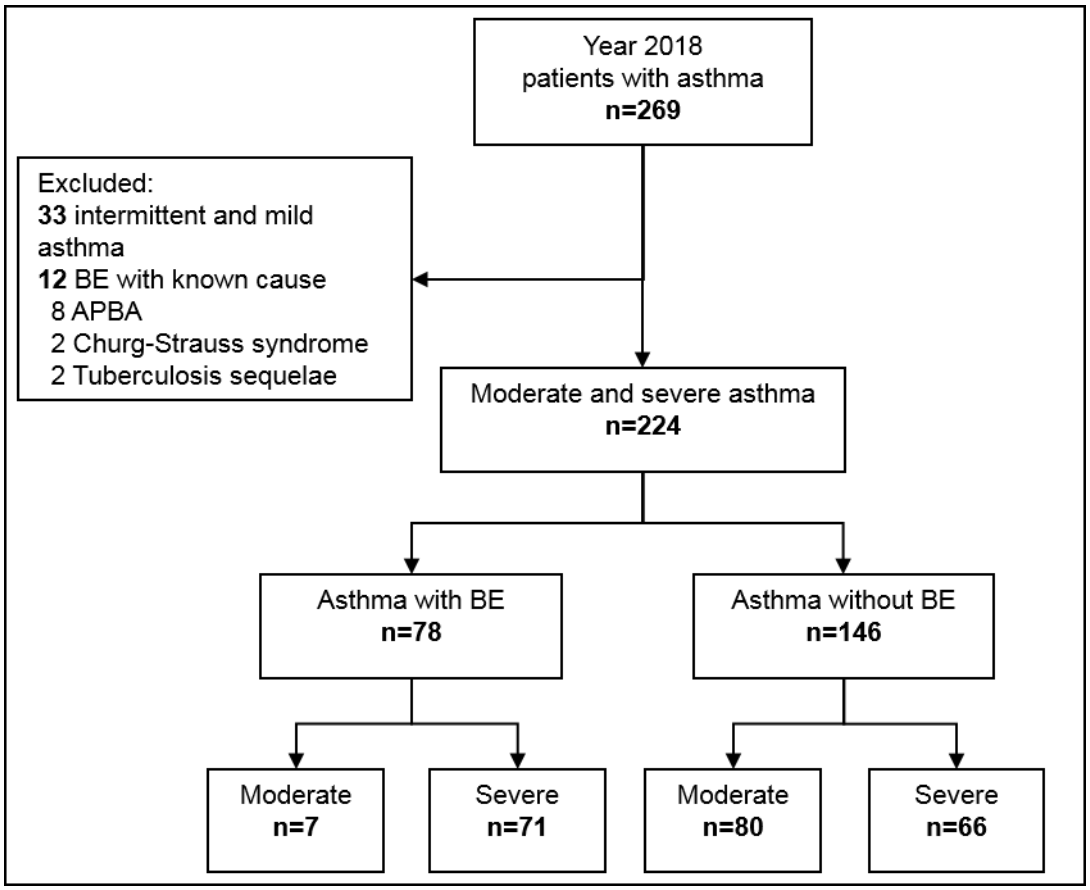

Figure 4.1.1. Flow chart of the study population. ABPA: allergic bronchopulmonary aspergillosis; BE: non-fibrosis bronchiectasis

In all the patients in the study, clinical history and data recorded including anthropometric, smoking habits, comorbidities, asthma history, asthma grade, atopy status and

exacerbations were retrospectively reviewed. Variables associated with bronchiectasis and microorganism colonization in sputum culture were also reviewed and recorded. Patients were examined and asked about their present medication, and the Asthma Control Test (ACT) (58) was administered. The records were checked to establish whether spirometry had been performed during the previous year or a High-Resolution Computed Tomography (HRCT) during the last three years. If not, or if no data were available, these tests were administered.

The study was approved by our hospital's Ethics Committee. All patients provided written informed consent to participate in the study.

Asthma diagnosis and severity

The diagnosis of asthma was made according to GINA (1) guidelines and based on clinical symptoms plus one or more complementary tests. All patients had shown reversible airway obstruction on methacholine challenge testing or bronchodilator testing, or variability > 20% in the peak flow recording. Moderate and severe asthma were defined based on the GINA guidelines, which corresponded to patients taking steps 3, 4, and 5 of medication with at least 200 µg of inhaled corticosteroids (budesonide or equivalent doses) plus a long-acting beta-2 agonist. (1)

Pulmonary function test

Spirometry was performed using a MasterLab instrument (MasterLab, Jaeger, Germany), according to European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines. (104) The reference values used were those proposed by the ERS. (105)

Bronchiectasis diagnosis and severity

Bronchiectasis was identified in accordance with the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) recommendations by comparing the internal bronchial lumen diameter with the adjacent artery calibre in high-resolution computed tomography images. (69)

Morphological characteristics of bronchiectasis, including bronchodilation, bronchial wall thickening, dilatation type and lobe extension were reviewed and scored according to the modified Reiff score (73) in six lobes (18 points in total): 1-6 mild, 7-12 moderate, and ≥13 severe.

The FACED parameter (74) was used for clinical estimation of the patients' status by

incorporating variables such as FEV1, age, pseudomonas colonization, lobe extension and dyspnea.

HRCT was performed with 1 mm cuts at 10 mm intervals in maximum inspiration. All the imaging features of bronchiectasis were interpreted by two pulmonologists individually. For controversial images, an expert radiologist was consulted and the final decision was made. The degree of the inter-observer agreement was assessed by the Kappa statistic. (106)

Atopy and smoking status

Patients were considered atopic if they had at least one positive prick test to any of the common environmental allergens. Non-smokers were patients who had never smoked, and ex-smokers were those who had not smoked for at least six months. The number of pack-years was calculated in all cases.

Definition of exacerbations

Episodes of asthma exacerbations during the previous year were recorded. Asthma exacerbations were defined according to GINA guidelines (1) which specify asthma attacks or acute asthma exacerbation as episodes of progressive increase in shortness of breath, cough, wheezing, or chest tightness, or some combination of these symptoms, accompanied by decreases in expiratory airflow.

Statistical analysis

Categorical variables were presented as frequencies and percentages. Statistical differences were analysed using the Chi-square test (or Fisher's exact test when appropriate). Continuous variables were presented as means and standard deviations (SD), or as medians with interquartile ranges (IQR) when data were not normally distributed. The Shapiro-Wilk test was used to analyse the distribution of variables. A logistic regression model was used to determine the factors that were independently associated with the outcome, in this case, the presence of bronchiectasis. Variables that presented statistically significant differences ($p<0.05$) in the bivariate analysis or were considered to be of clinical interest (such as gender) were included as independent variables in the first step. Variables associated with disease management and asthma control test score were not included. A forward stepwise technique (Wald test; removal threshold, $p>0.10$) was used to perform this analysis. ORs and 95% CIs were calculated for independent variables. Tolerance and the Variance Inflation Factor (VIF) were

examined to avoid multicollinearity among variables. Statistical significance was defined as a two-tailed $p \leq 0.05$. The statistical analyses were performed using SPSS (version 25, Chicago, IL).

RESULTS

The prevalence of bronchiectasis was 56.9% in severe asthma (Figure 4.1.1). Socio-demographic characteristics of patients with and without bronchiectasis (BE) are shown in table 4.1.1.

Table 4.1.1. Socio-demographic characteristics and clinical data of the study population (n=224).

	Moderate and severe asthma			**Severe asthma**		
	without BE (146)	with BE (78)	P	without BE (66)	with BE (71)	P
Sociodemographic data						
Gender (female)	94 (64.4%)	46 (59.0%)	0.43	37 (56.1%)	43 (60.6%)	0.59
Age (years)	49.9 (17.2)	56.2 (13.1)	**0.005**	49.7 (17.0)	56.2 (13.5)	**0.014**
Age group*			**0.004**			**0.033**
A1, <42 yrs	45 (30.8%)	9 (11.5%)	**<0.001**†	20 (30.3%)	9 (12.7%)	**0.009**†
A2, 42-65 yrs	65 (44.5%)	49 (62.8%)	0.364††	29 (43.9%)	43 (60.6%)	0.078††
A3, ≥65 yrs	36 (24.7%)	20 (25.6%)	0.023 ¶	17 (25.8%)	19 (26.8%)	0.491¶
Race (Caucasian)	141 (96.6%)	72 (92.3%)	0.20	63 (95.5%)	65 (91.5%)	0.50
BMI (kg/m^2)	27.1 (23.6,30.7)	27.1 (24.4, 29.1)	0.77	27.9 (24.7, 30.7)	27.3 (24.6, 29.1)	0.35
Smoking status			0.24			0.42
Non-smoker	100 (68.5%)	46 (59.0%)		43 (65.2%)	42 (59.2%)	
Current-smoker	8 (5.5%)	3 (3.8%)		4 (6.1%)	2 (2.8%)	
Ex-smoker	38 (26.0%)	29 (37.2%)		19 (28.8%)	27 (38.0%)	
Packs-year	0.0 (0.0, 3.0)	0.0 (0.0, 15.0)	0.052	0.0 (0.0, 4.0)	0.0 (0.0, 15.0)	0.30
Clinical data of asthma & lung function						
Atopic asthma	92 (63.0%)	34 (43.6%)	**0.005**	42 (63.6%)	30 (42.3%)	**0.012**
Asthma grade			**<0.001**			NA
Moderate	80 (54.8%)	7 (9.0%)				
Severe	66 (45.2%)	71 (91.0%)				
Asthma years	17.8 (9.4, 31.8)	19.4 (9.5, 37.1)	0.27	20.2 (13.3, 40.1)	20.4 (9.6, 37.3)	0.57
Asthma onset age	26.0 (6.0, 45.1)	33.7 (16.0, 45.9)	0.20	20.5 (5.5, 37.6)	33.8 (15.0, 46.0)	**0.024**
COA (yes)	49 (33.6%)	22 (28.2%)	0.41	25 (37.9%)	21 (29.6%)	0.30
ACT (pts)	21 (18, 24)	16 (13, 21)	**<0.001**	20.0 (17.0, 23.0)	16.0 (13.0, 21.0)	**0.003**
Exacerbation	0.0 (0.0, 1.0)	1.0 (0.0, 3.0)	**<0.001**	0.0 (0.0, 2.0)	2.0 (0.0, 4.0)	**<0.001**
≥3 courses	8 (5.5%)	27 (34.6%)	**<0.001**	8 (12.1%)	27 (38.0%)	**<0.001**
FVC% pred	89.4 (15.9)	82.8 (17.7)	**0.005**	86.8 (16.9)	81.8 (17.7)	0.096
FEV1% pred	81.0 (17.1)	73.2 (19.6)	**0.002**	75.8 (17.1)	72.2 (19.4)	0.25
FEV1/FVC	72.7 (9.0)	69.5 (9.1)	**0.014**	70.2 (8.5)	69.5 (9.2)	0.62
FEV1/FVC≥ 70	86 (58.9%)	42 (53.8%)	0.47	32 (48.5%)	34 (47.9%)	0.94
Comorbidity						
NSAIDs allergy	25 (17.1%)	13 (16.7%)	0.93	19 (28.8%)	11 (15.5%)	0.060
Rhinitis	50 (34.2%)	22 (28.2%)	0.36	21 (31.8%)	21 (29.6%)	0.78
Sinusitis	3 (2.1%)	8 (10.3%)	**0.018**	1 (1.5%)	8 (11.3%)	**0.034**
Nasal polyps	19 (13.0%)	22 (28.2%)	**0.005**	11 (16.7%)	20 (28.2%)	0.11
Obesity	46 (31.5%)	18 (23.1%)	0.18	23 (34.8%)	16 (22.5%)	0.11
WRA	25 (17.1%)	14 (17.9%)	0.88	10 (15.2%)	12 (16.9%)	0.78

	Moderate and severe asthma			Severe asthma		
	without BE (146)	with BE (78)	P	without BE (66)	with BE (71)	P
Disease management						
Cortisone-dependent	5 (3.4%)	27 (34.6%)	**<0.001**	4 (6.1%)	27 (38.0%)	**<0.001**
Budesonide (µg/d)	1040 (640,1600)	1280 (640,1600)	0.37	1440 (800,1600)	1400 (640,1600)	0.29
ICS dose category			0.14			0.61
Low	30 (20.5%)	10 (12.8%)		5 (7.6%)	9 (12.7%)	
Med	42 (28.8%)	18 (23.1%)		13 (19.7%)	14 (19.7%)	
High	74 (50.7%)	50 (64.1%)		48 (72.7%)	48 (67.6%)	
LAMA	39 (26.7%)	54 (69.2%)	**<0.001**	29 (43.9%)	53 (74.6%)	**<0.001**
Azithromycin	10 (6.8%)	26 (33.3%)	**<0.001**	8 (12.1%)	26 (36.6%)	**<0.001**
Omalizumab	18 (12.3%)	15 (19.2%)	0.16	18 (27.3%)	15 (21.1%)	0.40
Mepolizumab	4 (2.7%)	5 (6.4%)	0.28	4 (6.1%)	5 (7.0%)	1.00
Anti-leukotriene	57 (39.0%)	51 (65.4%)	**<0.001**	39 (59.1%)	48 (67.6%)	0.30
Theophylline	2 (1.4%)	1 (1.3%)	1.00	2 (3.0%)	1 (1.4%)	0.61

Continuous variables expressed as mean (SD) or median (p25, p75); categorical data expressed as percentage n (%); * Bonferroni adjust method was used to perform comparison between age subgroup, significant p level at 0.017; †, A1 vs A2; ††, A2 vs A3; ¶, A1 vs A3. BMI: body mass index; COA: childhood onset asthma (using 18 years as cut-off); ACT: asthma control test; BE: non-cystic fibrosis bronchiectasis; WRA, work-related asthma; NSAIDs, allergy to nonsteroidal anti-inflammatory drugs; LAMA: long-acting muscarinic receptor antagonists; ICS: Inhaled Corticosteroid. Significant p values in bold font.

Asthma patients with BE were older than patients without BE (56.2 vs. 49.9 years, p=0.005). Bronchiectasis was more frequent in middle-aged subjects (42-65 years) (62.8% vs 44.5%, p<0.05), but this trend was reversed in the younger adult group (<42 years) (11.5% vs 30.8%, p<0.05). Furthermore, subjects with BE had more severe asthma (91% vs 45.2%, p < 0.001) and presented a higher number of exacerbations (median 1 vs 0 episodes, p<0.001). Significantly lower FVC% pred, FEV1%pred and the ratio of FEV1/FVC were observed in asthma patients with BE than in those without BE (89.4% vs 82.8%, p=0.005; 81.0% vs 73.2%, p=0.002; 72.7 vs 69.5, p=0.014 respectively). Subjects with BE presented more sinusitis (10.3% vs 2.1%, p =0.018) and nasal polyps (28.2% vs 13.0%, p=0.005) than those without BE. Subjects without BE seemed to be more atopic than those with BE (63.0% vs 43.6%, p = 0.005). No significant differences were observed in the remaining comorbidities. With regard to disease management, subjects with BE were more corticosteroid-dependent (34.6% vs 3.4%, p < 0.001) and had also taken more azithromycin (34.6% vs 6.8%, p<0.001). In addition, the consumption of long-acting muscarinic receptor antagonists (LAMA) and anti-leukotriene was significantly higher in asthma patients with BE than in those without BE (69.2% vs. 26.7%, p < 0.001 and 65.4% vs. 39.0%, p < 0.001 respectively).

A sub-analysis limiting subjects to severe asthma was performed (Table 4.1.1). In this sub-analysis similar differences were found in age, ACT, asthma exacerbation, atopy, sinusitis, cortisone dependency and asthma medication (LAMA and azithromycin). However, the differences in lung function were no longer statistically significant. We further observed that patients with BE had a later asthma onset than patients without BE (median 20.5 years vs 33.8 years, p=0.024).

Of all the 78 patients with BE, 81% and 74% were classified as mild according to modified Reiff and FACED criteria respectively (Table 4.1.2). Patients with mild BE (FACED criterion) showed fewer years of asthma evolution than those with moderate and severe BE (17.2 yrs vs 31.7 yrs, p=0.037) (Figure 4.1.2). Seventy-five (96%) patients had widespread bronchial dilatation, with all six lobes involved. Bronchial wall thickening was moderate in 68% of patients and mild in 27%. The predominant dilatation type was cylindrical (82%). Kappa value (and overall agreement %) for the lobe extension, bronchial dilatation, bronchial wall thickening, and dilatation type were 0.83 (98.7%), 0.75 (88.5%), 0.70 (89.7%), and 0.79 (94.9%) respectively.

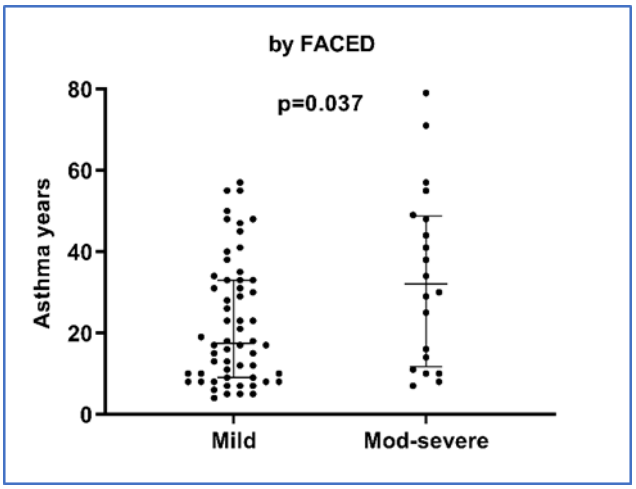

Figure 4.1.2. Asthma-year according to bronchiectasis severity assessed by FACED.
Data expressed as median (IQR). Asthma year 17.2 (9.1, 32.5) in mild vs 31.7 (12.0, 48.3) in moderate and severe bronchiectasis, p=0.037.

Table 4.1.2. Assessment of bronchiectasis by radiological and clinical parameters.

Comprehensive parameter		Individual parameter	
		Lobe extension with lingula included	
FACED		4 lobes involved	3 (4%)
		6 lobes involved	75 (96%)
Mild	58 (74%)	**Bronchial wall thickening* (predominant)**	
Moderate	18 (23%)	Mild	21 (27%)
Severe	2 (3%)	Moderate	53 (68%)
		Severe	4 (5%)
Modified Reiff		**Dilatation type (predominant)**	
Mild	63 (81%)	Cylindrical	64 (82%)
Moderate	13 (17%)	Varicose	12 (15%)
Severe	2 (3%)	Cystic	2 (3%)

Left part: systemic parameters. Right part: individual parameters. * Bronchial wall thickening by comparing with the adjacent artery diameter: mild< 0.5 A; moderate (0.5-1) A; severe> 1A. Modified Reiff score:1-6 pts mild, 7-12 pts moderate and ⩾13 pts severe. FACED score: 0-2 pts mild, 3-4 pts moderate and 5-7 pts severe bronchiectasis.

Potentially pathogenic bacteria were identified in 16 (21%) subjects with BE, including P. aeruginosa (5 cases), H. influenzae (3 cases), S. pneumonia (3 cases), S. aureus (2 cases), K. pneumonia (1 case), M. catarrhalis (1 case) and P. putida (1 case).

The odds ratios and 95% confidence intervals of variables related to bronchiectasis in all patients and in severe asthma patients are shown in Figures 4.1.3 and 4.1.4 respectively. Age, severe asthma grade and asthma exacerbations (≥3) were independently associated with the presence of bronchiectasis in patients with moderate and severe asthma. In subjects with severe asthma alone, age of asthma onset and ≥3 exacerbations were independent factors associated with the same outcome.

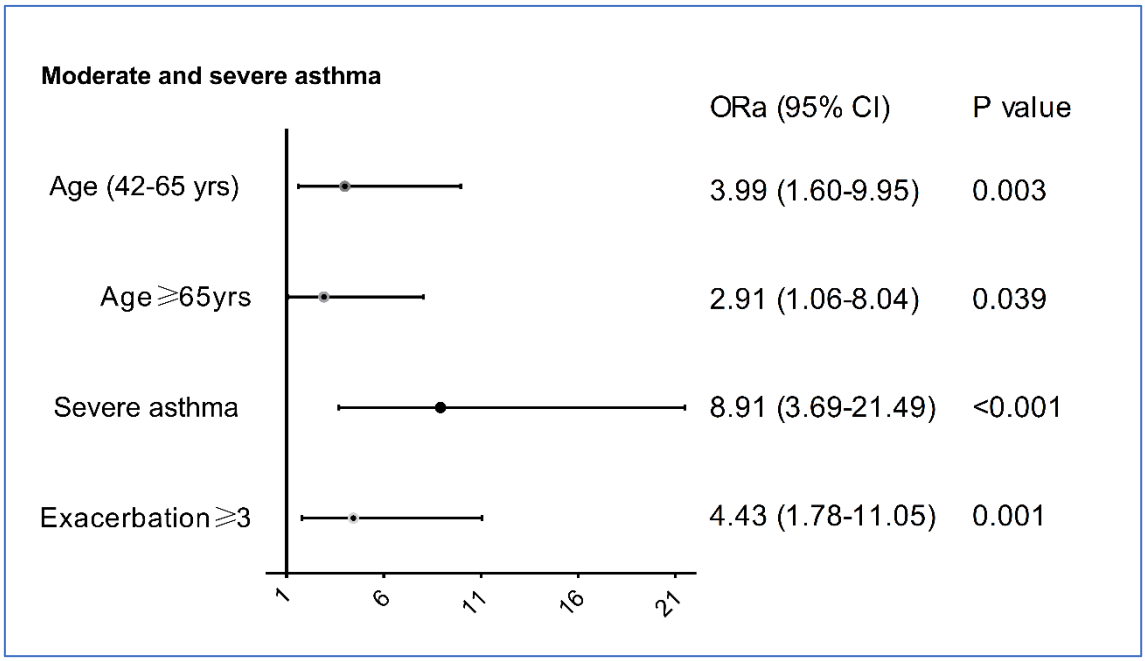

Figure 4.1.3. Factors associated with bronchiectasis in all subjects (moderate and severe asthma).

ORa: adjusted odds ratio. CI: confidence interval. Model: $\chi 2=73.64$, $p<0.001$. Model adjusted by gender, atopy, sinusitis, nasal polyps, FVC% and FEV1%. Logistic regression was used to perform the analysis.

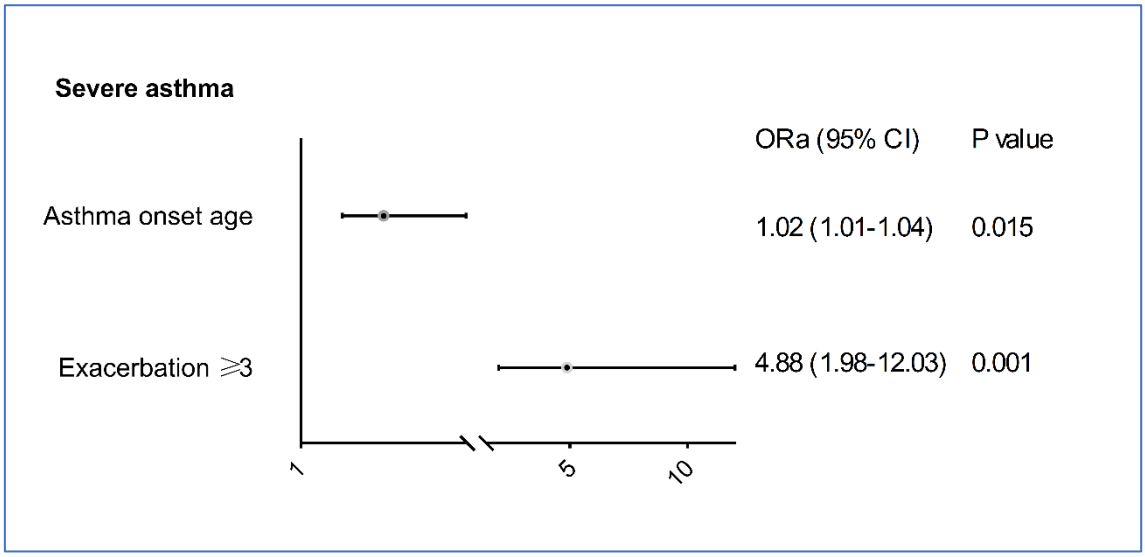

Figure 4.1.4. Factors associated with bronchiectasis in severe asthma patients.

ORa: adjusted odds ratio. CI: confidence interval. Model: $\chi 2=18.82$, $p<0.001$. Model adjusted by gender, age group, atopy and sinusitis. Logistic regression was used to perform the analysis.

4.2. Study 2. Expression of proinflammatory and remodelling cytokines in asthma patients with and without bronchiectasis.

Study population and design

Severe asthma patients derived from study 1 were asked to make a sputum induction for the further study of inflammatory phenotype and cytokine expression. (Figure 4.2.1) Sputum samples with guaranteed quality were collected from 55 severe asthma patients with bronchiectasis (group AB) and 45 without bronchiectasis (group AC). Differential cell counts (eosinophils, neutrophils, macrophages and lymphocytes) were performed to determine asthma inflammatory phenotypes (eosinophilic and neutrophilic). Sputum supernatants were analysed to compare cytokine profile.

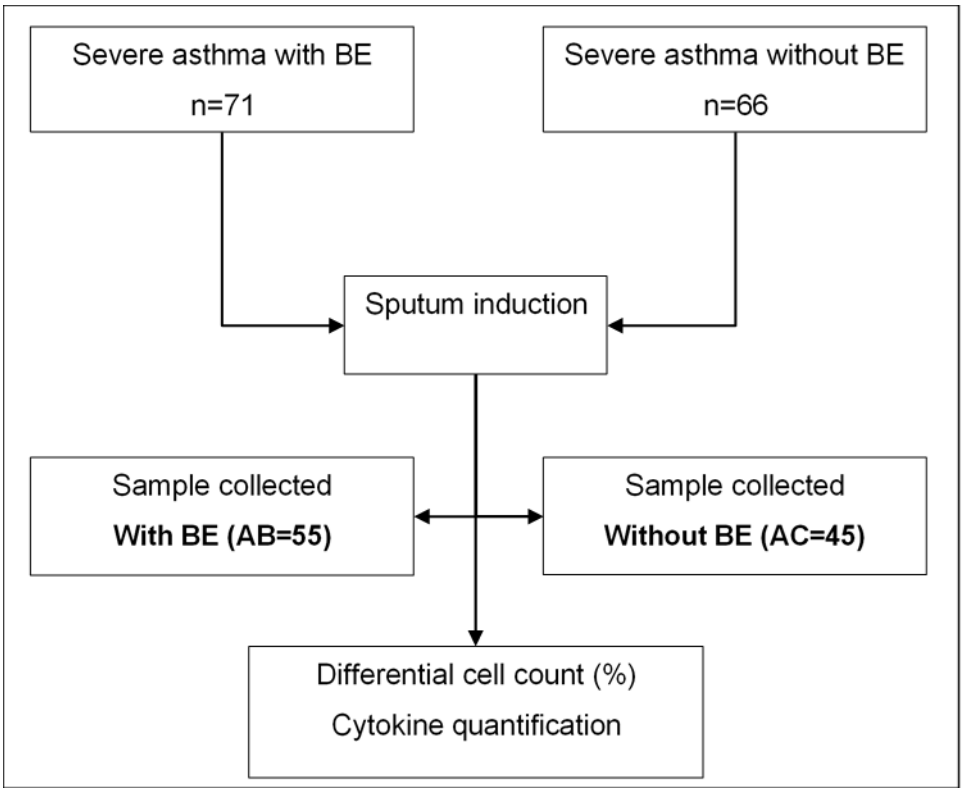

Figure 4.2.1. Flow chart and study design. BE, Bronchiectasis.

The study was approved by our hospital's Ethics Committee. All patients provided written informed consent prior to participating.

Definition of eosinophilic and neutrophilic inflammation

The type of inflammation was determined by sputum differential cell count based on the criteria of Nair and Ray. (31,107) The neutrophilic phenotype was defined by the presence

of ≥65% neutrophils and <3% eosinophils; the eosinophilic phenotype by ≥3% eosinophils and <65% neutrophils; the mixed phenotype by the presence of ≥3%, eosinophils and ≥65% neutrophils, and the paucigranulocytic phenotype by both <3% eosinophils and <65% neutrophils.

Questionnaires

Questionnaires including the Asthma Control Test (ACT) (58), Hospital Anxiety and Depression Scale (HADs) (108), Mini Asthma Quality of Life Questionnaire (MiniAQLQ) (109) and the modified Medical Research Council Dyspnea Scale (mMRC) (110) were completed during the study. (See annex)

Sputum induction and processing

Induced sputum was obtained using an ultrasonic nebulizer (OMRON, Hoofddorp, Netherlands) with an output of 1 ml/min at room temperature (Figure 4.2.2). All patients were instructed to blow their nose and rinse their mouth thoroughly to minimize the saliva contamination. The test was completed by inhalation of a stepwise hypertonic solution (3%, 4% and 5%); subjects breathed through their mouths for 7 minutes, keeping the nose clipped. A deep cough and expectoration were indicated after each exposure to facilitate the collection of sputum samples. In all cases, the induction procedures were only performed if the FEV1 was more than 1.5 litre. (Figure 4.2.3)

Figure 4.2.2. Ultrasonic nebulizer.

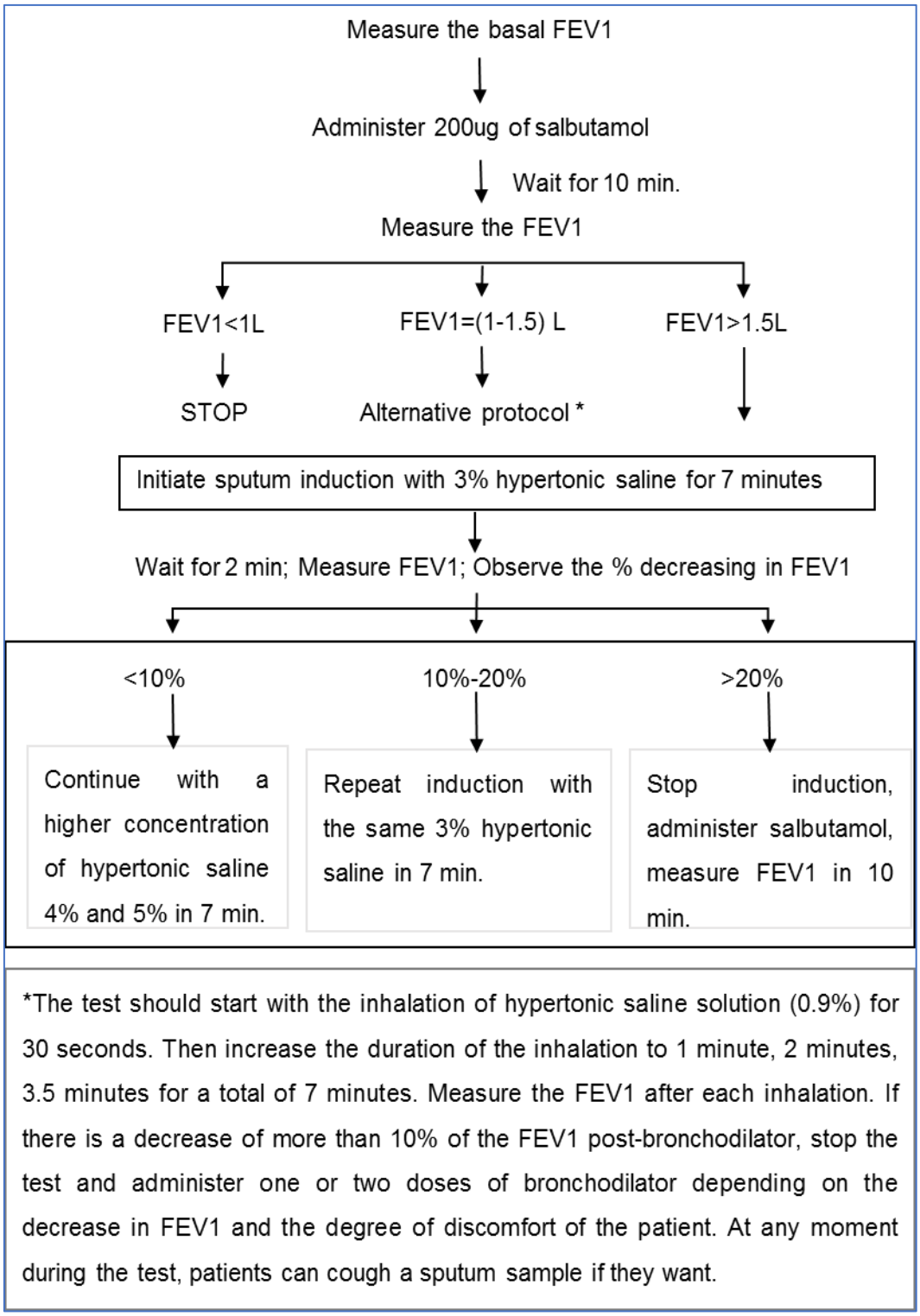

Figure 4.2.3. Algorithm of the sputum induction method.

The sputum samples obtained were processed immediately using the previously standardized protocol. (111,112) In summary, the selected expectorate sputum sample was firstly weighed and treated with four times the volume of a working solution containing 0.1% dithiothreitol (DTT) (SIGMA-ALDRICH) and then gently vortexed for 10 minutes. A

further fourfold volume of PBS buffer was added to stop the effect of DTT on the cell suspension. The sample solution was filtered through a 40mm nylon gauze (FALCON). A small portion (12 µl) was collected to determine the cell viability in a Neubauer camera using the trypan blue exclusion method. The rest of the sample was centrifuged at 1000 x g 4℃ for 10 minutes to separate the supernatant from the cell pellet. Cell pellet was resuspended, two slides were prepared by cytospin (Shandon) and were stained with May-Grunwald and Giemsa for differential cell counts by counting at least 400 non-squamous cells. (Figure 4.2.4) The supernatant was aliquoted and stored at – 80℃ for later assay.

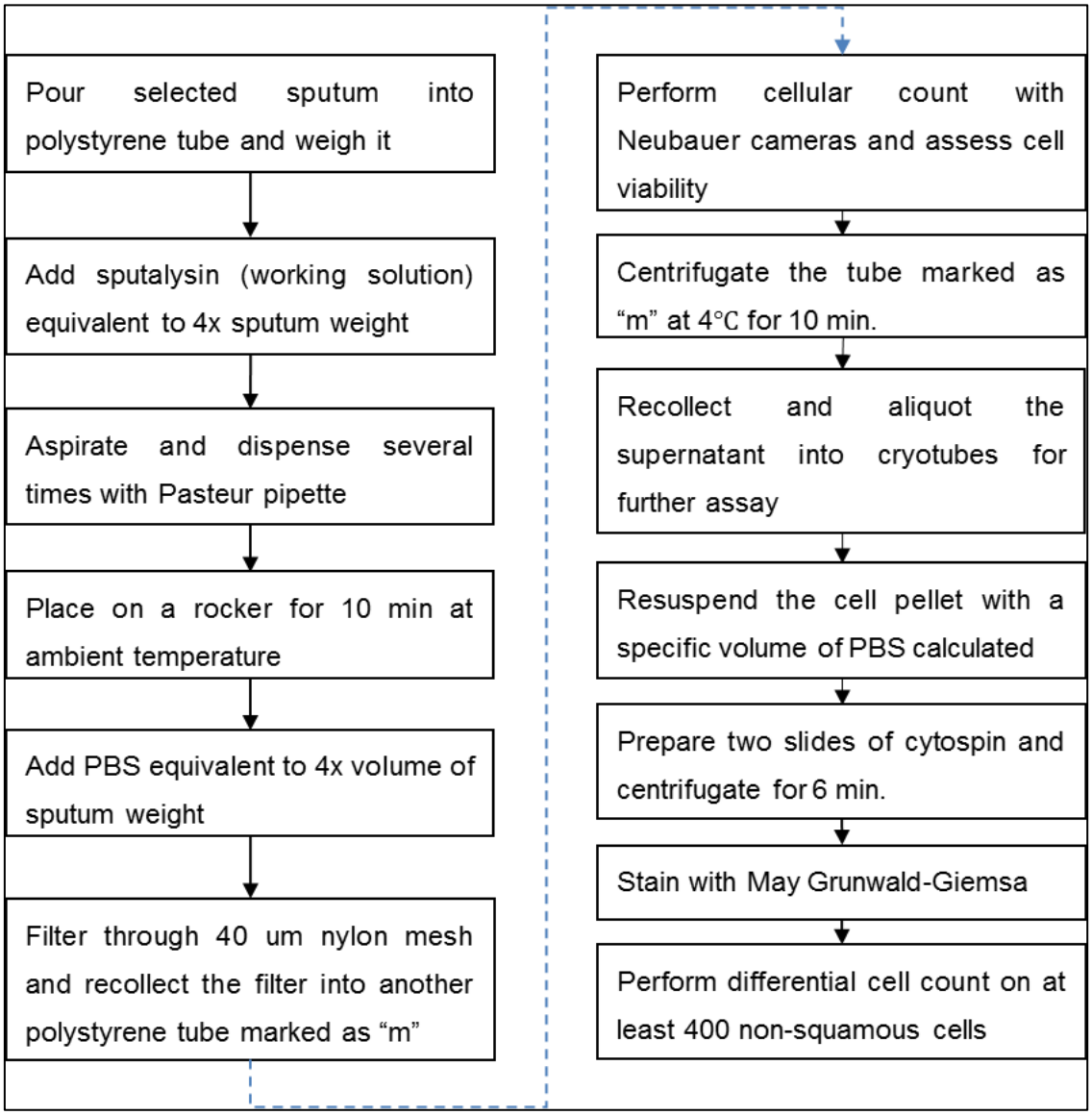

Figure 4.2.4. Summary of sputum processing scheme; PBS: Phosphate Buffered Saline.

Quantification of biomarkers in sputum supernatant

Inflammatory and proinflammatory cytokines: TNF-α, IFN-γ, GM-CSF (Bio-Rad Lab, Inc), IL8 (R&D Systems, Inc), airway remodelling-related cytokines: TGFβ1 and VEGF (R&D Systems, Inc), and cytokines related to bronchiectasis: PMN elastase (Invitrogen) were measured. All the above cytokines were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Since the levels of IL5 and IL13 were undetectable in our pilot test, these two biomarkers were not analysed in the later assay.

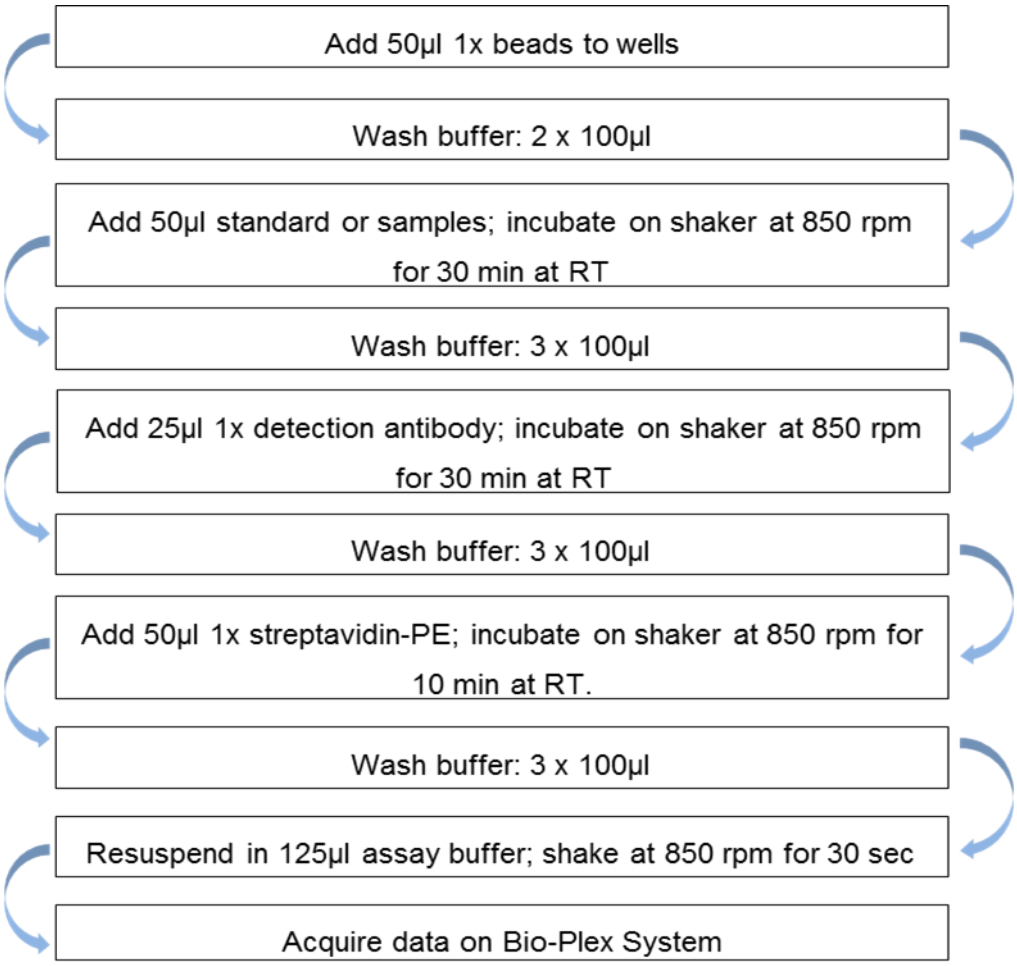

Figure 4.2.5. A quick assay protocol for the bio-plex immunoassay technique.

Statistical analysis

Statistical analysis was performed using Stata version 15.1 (StataCorp; Texas; USA). Variable normality was tested by the Kolmogorov–Smirnov test. Categorical variables are presented as n (%), and continuous variables as mean (SD) or median (P25, P75) according to data distribution. Comparisons between groups were performed using the

chi-square test for qualitative variables or the Fisher exact test when appropriate. Student's t-test or Wilcoxon rank-sum test was used to compare continuous variables between groups according to the normal or non-normal distribution of the data. The significance of correlations was evaluated by determining Spearman rank correlation coefficients. A p-value<0.05 was considered statistical significance.

RESULTS

The social-demographic and functional data of the study participants are shown in table 4.2.1. In both groups the female population predominated, with median ages of 52 and 56 years in AB and AC groups respectively. No significant differences were found in gender and smoking status between the cohorts. A large proportion of patients showed intolerance to nonsteroidal anti-inflammatory drugs (NSAIDs) in the AC group (38% vs 18%, p=0.028), and presented atopic asthma (62% vs 38%, p=0.017). We observed that subjects in the AB group more frequently presented asthma exacerbation (median 2 vs 0 courses, p<0.001). Forty-two (76%) patients experienced at least one asthma exacerbation in the prior year, significantly more than in the AC group (p=0.002). Furthermore, patients in the AB group had worse asthma control than controls (16 pts vs 21 pts, p=0.017). No significant differences were observed with regard to lung function. Additionally, a larger proportion of patients were considered as oral corticosteroid-dependent in the AB group (42% vs 4%, p<0.001), these patients were also more likely to take LAMA (77% vs 44%, p=0.007) and azithromycin (36% vs 11%, p=0.005).

Table 4.2.1. Socio-demographic and clinical data and questionnaires.

	AC **n = 45**	**AB** **n = 55**	p-value
Gender, female	27 (60%)	33 (60%)	1.00
Age (yrs)	52 (16)	56 (13)	0.1
Race (Caucasian)	42 (93%)	51 (93%)	1.00
BMI (kg/m^2)	28 (26, 31)	27 (25, 29)	0.17
Smoking habit			0.83
Non-smoker	28 (62%)	31 (56%)	-
Current smoker	2 (4.4%)	2 (3.6%)	-
Ex-smoker	15 (33%)	22 (40%)	-
Atopic asthma	28 (62%)	21 (38%)	**0.017**
Intolerance to NSAIDs	17 (38%)	10 (18%)	**0.028**
Asthma years	26 (14, 40)	23 (10, 37)	0.54
Asthma onset age	20 (6, 40)	33 (17, 44)	0.13
Childhood-asthma	16 (36%)	15 (27%)	0.37
Cough (yes)	21 (48%)	28 (51%)	0.84
Expectoration (yes)	12 (27%)	20 (36%)	0.39
Wheezing (yes)	37 (84%)	32 (58%)	**0.008**
mMRC dyspnea			0.10
0	7 (16%)	5 (9.1%)	-

	AC n = 45	AB n = 55	p-value
1	24 (53%)	23 (42%)	-
2	8 (18%)	22 (40%)	-
3	6 (13%)	5 (9.1%)	-
Exacerbation times	0 (0, 2)	2 (1, 5)	**<0.001**
≥1	21 (47%)	42 (76%)	**0.002**
≥2	13 (29%)	34 (62%)	**0.001**
≥3	7 (16%)	26 (47%)	**<0.001**
Emergency visits times	0 (0, 0)	0 (0, 2)	**<0.001**
ACT (pts)	21 (17, 23)	16 (12, 22)	**0.017**
ACT<20 pts	20 (44%)	37 (67%)	**0.022**
FVC% pred.	87 (17)	85 (15)	0.46
FEV1% pred.	78 (17)	76 (17)	0.50
FEV1/FVC	71 (8.7)	70 (8.9)	0.56
FEV1/FVC< 70	20 (44%)	23 (42%)	0.79
DLCO% pred.	80 (16)	78 (16)	0.65
KCO% pred.	83 (13)	81 (14)	0.67
RV% pred.	133 (36)	121 (45)	0.23
TLC% pred.	103 (15)	101 (16)	0.64
RV/TLC	44 (12)	41 (15)	0.43
FeNo (ppb)	25 (11, 47)	28 (8, 50)	0.78
IgE (U/ml)	301 (127, 879)	177 (67, 575)	0.19
AQLQ score (pts)	5.4 (3.8, 6.1)	4.6 (3.2, 5.7)	0.067
Anxiety (yes)	17 (38%)	28 (51%)	0.19
Depression (yes)	11 (24%)	17 (31%)	0.47
Cortisone-dependent	2 (4.4%)	23 (42%)	**<0.001**
Budesonide (µg/d)	1440 (1040, 1600)	1400 (640, 1600)	0.36
LABA	45 (100%)	55 (100%)	-
LAMA	20 (44%)	39 (71%)	**0.007**
Anti-leukotriene	28 (62%)	33 (60%)	0.82
Theophylline	0 (0%)	1 (1.8%)	1.00
Azithromycin	5 (11%)	20 (36%)	**0.005**
Omalizumab	14 (31%)	8 (15%)	**0.047**
Mepolizumab	3 (6.7%)	5 (9.1%)	0.73

AC: asthma without bronchiectasis; AB: asthma with bronchiectasis; Continuous variables expressed as mean (SD) or median (p25, p75); categorical variables in n (%); mMRC: modified Medical Research Council; HAD: Hospital Anxiety and Depression Score; mini-AQLQ: mini Asthma Quality of Life Questionnaire. HADa: HAD anxiety score; HADd: HAD depression

score. *Anxiety and depression were defined using a cut-off value of 7 points. ≤7 normal, ≥8 abnormal. NSAIDs: nonsteroidal anti-inflammatory drugs; LABA: Long-Acting Beta-Agonists; LAMA: long-acting muscarinic antagonist. Significant p value in bold font.

No differences were observed in eosinophils and neutrophils either in peripheral blood or in the sputum sample. Of the 55 asthma subjects with bronchiectasis, 20 (36%) had neutrophilic asthma, 15 (27%) had eosinophilic asthma, 10 (18%) had paucigranulocytic asthma and 10 (18%) had a mixed inflammation. In the control group the neutrophilic inflammation also predominated (40%) (Table 4.2.2). There were no significant differences in either the percentage of eosinophils and neutrophils or the subtype of inflammation observed (neutrophilic or eosinophilic) between the two groups (Table 4.2.2).

Table 4.2.2. Inflammatory profile and phenotype in patients with bronchiectasis (AB) and without bronchiectasis (AC).

	AC (45)	**AB (55)**	p-value
Blood			
Leucocyte, x10^9/L	7.9 (6.5, 9.2)	8.6 (7.1, 11)	0.13
Neutrophil, %	56 (49, 64)	59 (52, 69)	0.12
Lymphocyte, %	30 (24, 36)	28 (20, 34)	0.13
Monocyte, %	6.9 (6.1, 8.8)	7.7 (6.5, 9.5)	0.19
Eosinophil, %	4.4 (2, 6.4)	3.4 (1.6, 5.8)	0.23
Basophil, %	0.7 (0.5, 0.9)	0.6 (0.4, 0.8)	0.05
Absolute eos. (cell/µl),	300 (200, 500)	300 (100, 500)	0.47
Sputum			
Sputum appearance			0.15
Mucoid	38 (84%)	39 (71%)	-
Mucopurulent	3 (6.7%)	11 (20%)	-
Purulent	4 (8.9%)	5 (9.1%)	-
Total cells, 10^6/ml	0.52 (0.1, 2)	0.93 (0.26, 3.2)	0.20
Eosinophil, %	1.8 (.3, 7.6)	1.6 (0, 18)	0.74
Neutrophil, %	75 (49, 84)	69 (46, 83)	0.57
Lymphocyte, %	0.8 (0.4, 1.3)	0.8 (0.2, 1.5)	0.94
Macrophage, %	17 (9.8, 32)	21 (7.4, 30)	0.91
Phenotype*			0.89
Eosinophilic	10 (22%)	15 (27%)	-
Neutrophilic	18 (40%)	20 (36%)	-
Paucigranulocytic	7 (16%)	10 (18%)	-

	AC (45)	AB (55)	p-value
Mixed	10 (22%)	10 (18%)	-

AC: asthma without bronchiectasis; AB: asthma with bronchiectasis; Continuous data expressed as median (p25, p75); categorical variables appear in n (%) *eosinophilic, eos≥3%; neutrophilic, neu≥65%; mixed, both eos≥3% & neu≥65%; paucigranulocytic, both eos<3% & neu<65%.

Increased levels of TGFβ1, VEGF and IFNγ were observed in the AB group compared to the AC group (15 vs 24 pg / ml, p = 0.014; 183 vs 272 pg / ml, p = 0.048; 0.85 vs 19 pg / ml, p <0.001, respectively). The level of GM-CSF decreased significantly in the AB group (1.2 vs. 4.4 pg / ml, p <0.001). Levels of IL-8, neutrophil elastase and TNFα did not show significant differences between the groups (Figure 4.2.6).

A significant correlation was found between sputum eosinophil percentage and the blood absolute eosinophils in the AB group (rs=0.67, p<0.001), as well as in the AC group (rs =0.41, p<0.01). (Figure 4.2.7).

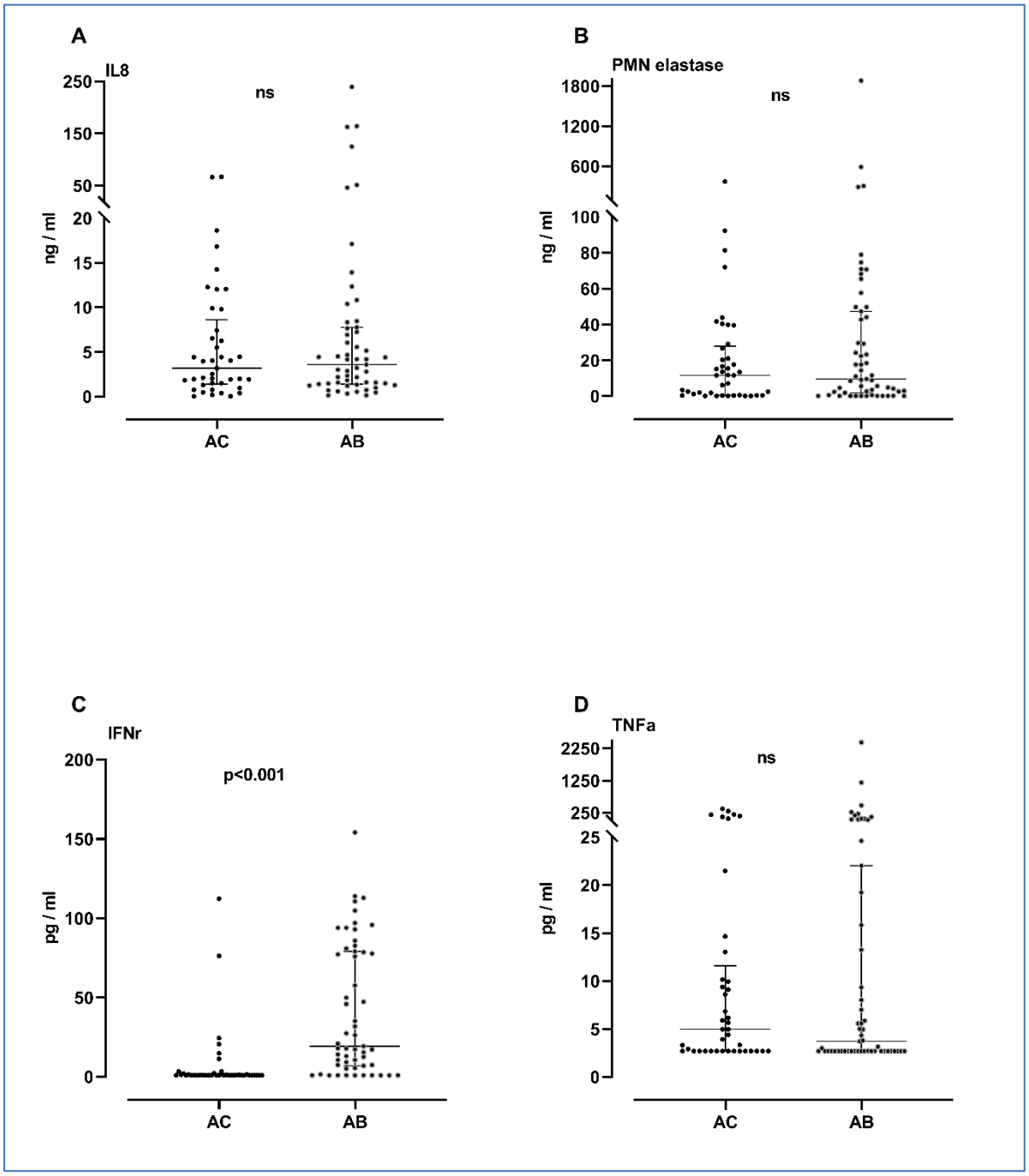

Figure 4.2.6. Pro-inflammatory and airway remodelling cytokines. A: Interleukin 8 (IL-8); B: human neutrophil elastase; C: interferon gamma (IFN-γ); D: tumour necrosis factor alpha (TNF-α); Data expressed as median and interquartile range.

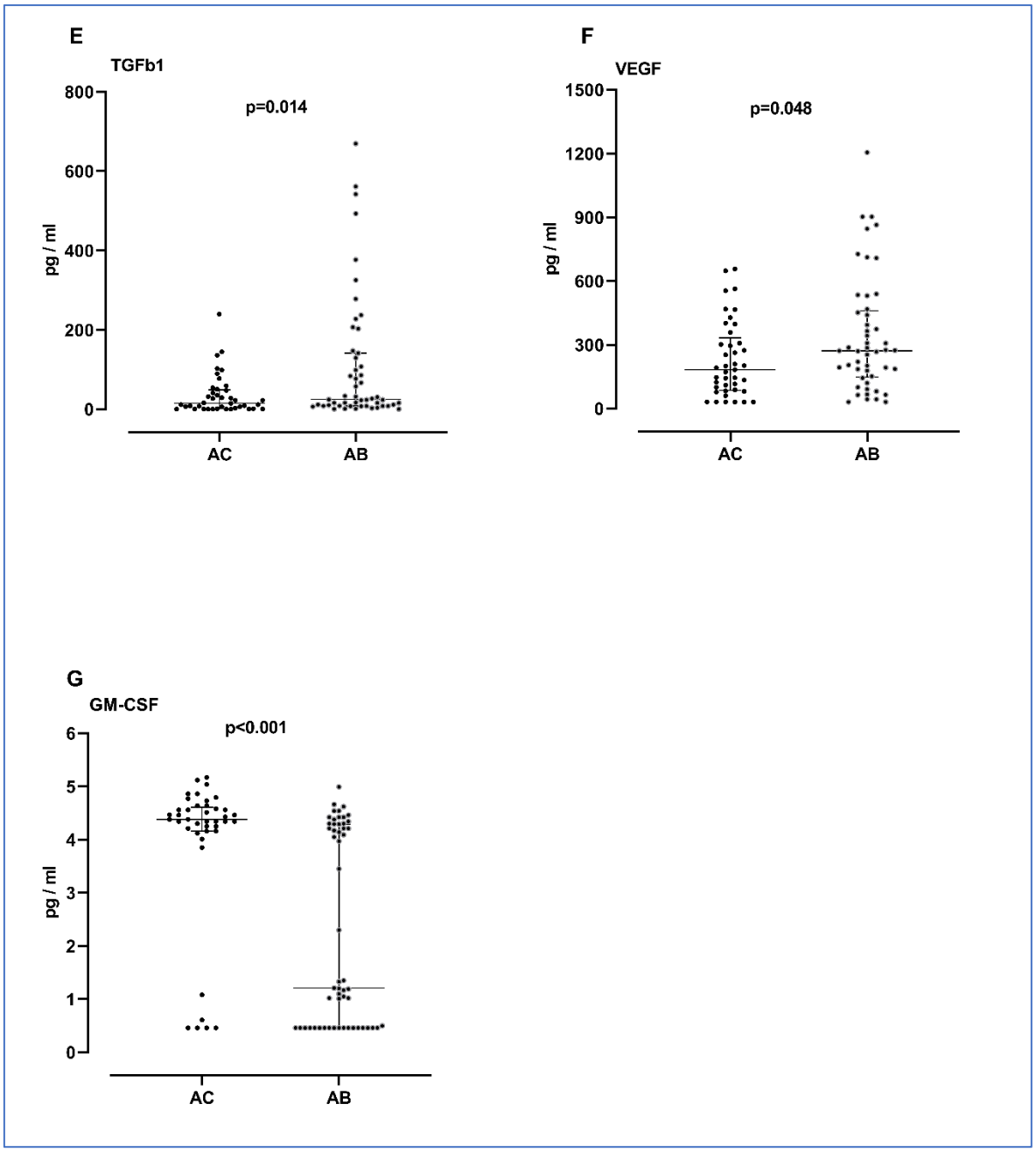

Figure 4.2.6 Continued. Pro-inflammatory and airway remodelling cytokines. E: transforming growth factor-beta 1; F: vascular endothelial growth factor (VEGF); G: granulocyte-macrophage colony-stimulating factor (GM-CSF). Data expressed as median and interquartile range.

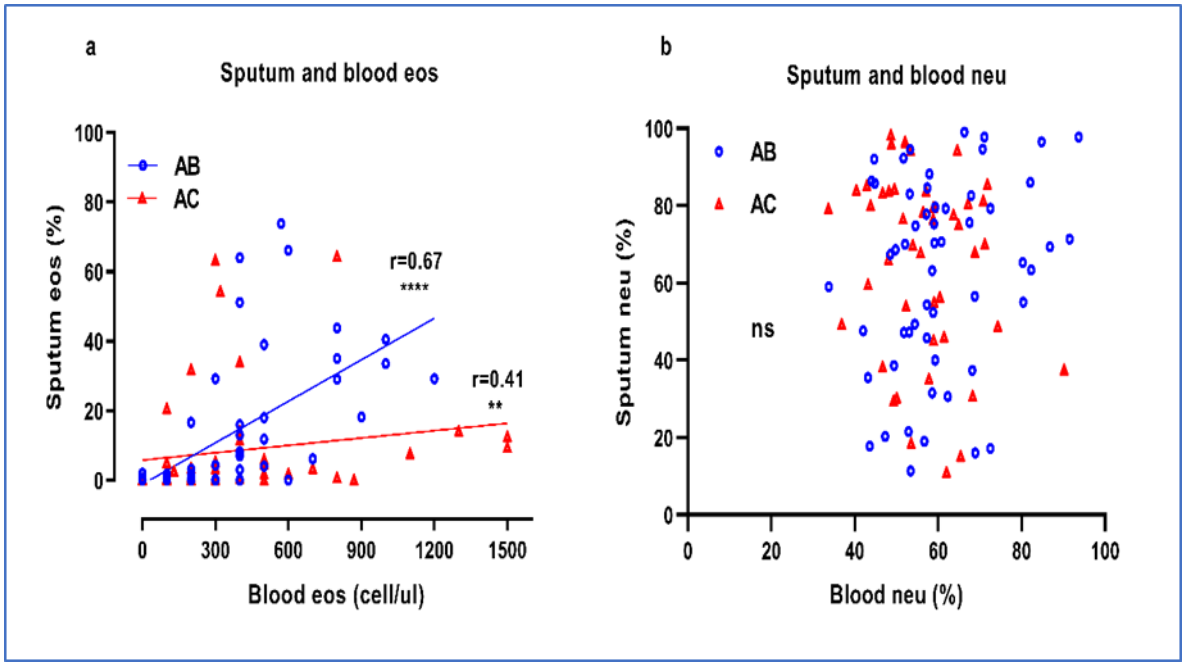

Figure 4.2.7. Spearman correlation between blood and sputum inflammatory cells. a) eosinophils; b) neutrophils. AC: without bronchiectasis; AB: with bronchiectasis.

As shown by the correlation matrix in Figure 4.2.8, among IL8, TNFα, neutrophil elastase, TGFβ1 and VEGF level presented a moderate positive correlation regardless of the presence of bronchiectasis. IFNγ presented a negative correlation with GM-CSF in the AB group (rs=-0.64, p<0.001). A moderate correlation was observed between TGFβ1 and VEGF in the AC group (rs=0.50, p<0.001).

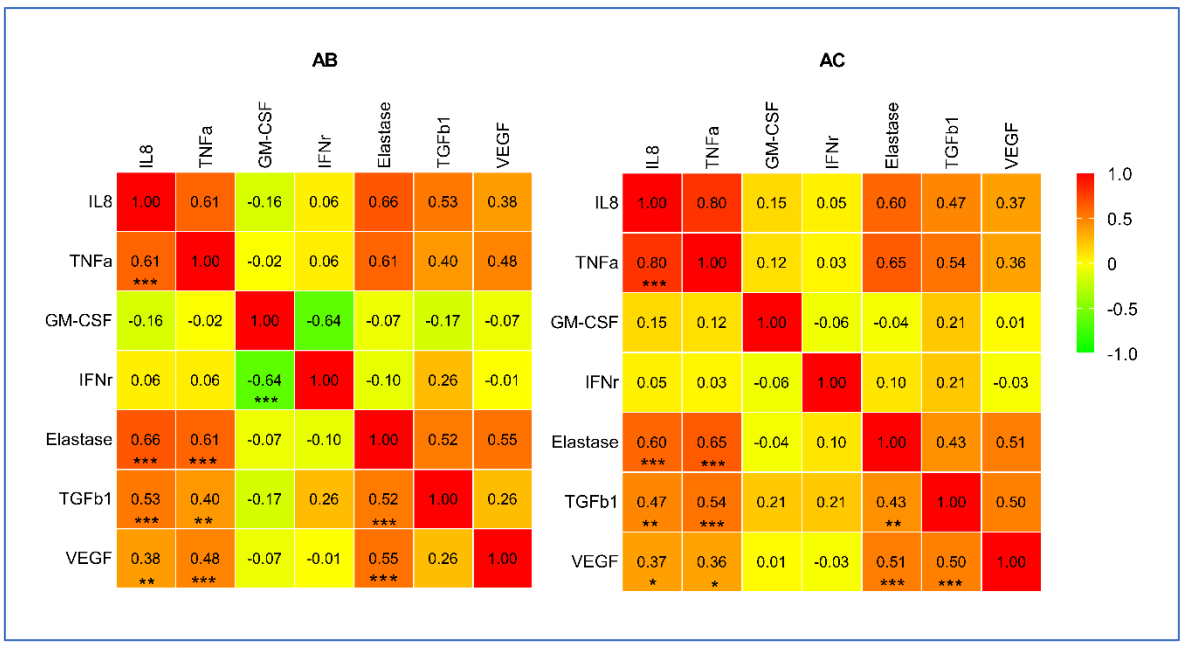

Figure 4.2.8. Spearman-correlation matrix of studied cytokines. Each coloured square represents the correlation between two cytokines. Red indicates a strong positive correlation, and green a strong negative correlation. Significant p values are marked in the lower triangle. *P < 0.05, **P < 0.01, and ***P < 0.001. AC: without bronchiectasis; AB: with bronchiectasis.

DISCUSSION

5. DISCUSSION

5.1. Discussion of study 1

In the present study a high percentage of bronchiectasis in asthma patients was found, especially in those with severe asthma. Independent factors associated with the presence of bronchiectasis were also assessed, demonstrating that, in severe asthma patients, age of asthma onset and exacerbations were independent factors associated with the occurrence of bronchiectasis.

Though asthma and bronchiectasis are two different diseases, their coexistence has been shown in many patients. In 2012, in a study applying High-Resolution Computed Tomography (HRCT) to persistent moderate asthma patients Khadadah et al (84) reported the presence of bronchiectasis in 28.6% of subjects. In patients with persistent severe asthma, the presence of bronchiectasis was higher and ranged between 35%-80% according to study. (85–88,90,91,113) Similar to our results, in a large cohort of asthma patients Oguzulgen et al (80) recently reported that most asthma patients with bronchiectasis had severe persistent asthma (49.0%). In pediatric populations with severe asthma, approximately one-third of children had bronchiectasis, which was associated especially with older children. (114) The differences observed between the studies may have been due to the study design or to the heterogeneity of the study populations and comorbidities. Moreover, the wide use of HRCT has already contributed to the increases in the prevalence of radiological bronchial dilatation and other airway abnormalities. In this regard, data from the US reported that the performance of HRCT has risen from 3 million patients in 1980 to 81.2 million in 2014. (115)

As we mentioned above, the coexistence of bronchiectasis and asthma has been observed in many patients, but few studies have been undertaken to investigate the relationship between the two diseases and the effect on exacerbations. Analysing the effects of asthma on bronchiectasis exacerbation, Mao et al (116) found that the presence of asthma was associated with an independent increase in the risk of bronchiectasis exacerbation in patients with non-cystic fibrosis bronchiectasis. Kang et al (117) assessed the impact of bronchiectasis on asthma and found that bronchiectasis may be a risk factor for asthma exacerbation; these authors suggested that, HRCT could be considered for concurrent bronchiectasis in uncontrolled asthma patients. In fact, bronchiectasis may be a terminal status in some patients with chronic airway inflammation, and early detection of bronchiectasis is recommended when patients present these features. Crimi et al (118)

also advocate the use of HRCT to rule out bronchiectasis in patients with severe asthma and frequent exacerbations. A 5-year follow-up study reported a mortality rate of 20% in adults with bronchiectasis. (119) Bronchiectasis challenges asthma control and may aggravate the evolution of the condition.

Although asthma severity is an independent risk factor for bronchiectasis, the question of whether it is a cause of bronchiectasis remains unclear. In mild to moderate predominant bronchiectasis, asthma was the cause in 5.4% of cases. (120) Moreover, a Finnish study suggested that one-fourth of cases with bronchiectasis could be attributed to asthma. (121) The British guideline also recommends considering asthma as the cause of bronchiectasis in the absence of any other etiological explanation. (93)

There are several reasons why exacerbations can occur in these patients. Asthma exacerbations are associated with a variety of risk factors including allergens, pollutants, drugs, obesity, emotional stress, rhinitis, sinusitis, gastro-esophageal reflux and menstruation, but respiratory infections (viral and bacterial) are considered among the major triggers. In this regard, in asthma patients with bronchiectasis, persistent bacterial colonization and airway inflammation related to the presence of bronchiectasis may be associated with exacerbations. Of all the potential pathogenic microorganisms, P. aeruginosa accounts for between 20 and 40% of cases. (79) The results of Chalmers et al's study corroborated our findings, given that bacterial colonization was identified in one-fifth of subjects with bronchiectasis. Other conditions such as autoimmune and immune defence deficit disease also make subjects more susceptible. (122) Moreover, airway remodelling is quite frequent in severe asthma patients. The destruction of the normal airway and the hyperproliferation of fibroblasts leads to the accumulation of mucus, facilitating recurrent bacterial colonization and further triggering new courses of asthma exacerbation, as in the classical vicious circle theory described by Cole et al. (95)

Bronchodilatation and bronchial wall thickening are very common in severe asthma patients, (123–125) but there are fewer studies giving systematic quantification scores of these abnormalities in asthma patients. The present study describes the characteristics and severity of bronchiectasis, showing mainly a mild grade of bronchiectasis according to the criteria of Reiff (73) and FACED (74). Our results are consistent with the study by Padilla-Galo et al (83) on the severity of bronchiectasis, in which the mean FACED score was 1.45 pts, also corresponding to mild grade disease. In contrast, they observed a mild predominant grade of bronchial wall thickening, while we found a notable proportion of patients with moderate predominant wall thickening. This difference may be attributed to

the different degrees of asthma severity analysed in the two studies. In our study 82% of patients presented cylindrical dilatation, Padilla-Galo et al reported a similar rate (92.9%).

We found that patients with asthma and bronchiectasis were more likely to present sinusitis and nasal polyps. The association of sinusitis, nasal polyps and intolerance to aspirin, known as Samter's Triad Syndrome, (126) is a common phenomenon in patients with asthma and a relationship between asthma severity and nasal polyps has been reported, (127) but the pathology of bronchiectasis and its interaction with other nasal airway diseases requires further exploration. Nonetheless, in patients with bronchiectasis, nasal polyps and chronic sinusitis are quite frequent and are also characterized by early onset. (128,129) In fact, chronic sputum expectoration is common in patients with bronchiectasis and the mucus hypersecretion has been associated with rhinosinusitis and nasal polyps. (130) The present study also found that asthma patients with bronchiectasis were less atopic. In this connection, atopy was significantly more prevalent in patients with asthma than in patients with chronic rhinosinusitis and nasal polyps. (126) Likewise, atopic dermatitis is less frequent in subjects with bronchiectasis than in those without (2% vs 11%, OR 0.188). (113)

In patients with moderate and severe asthma, the present study found that the factors independently associated with the occurrence of bronchiectasis were older age, asthma severity and frequent asthma exacerbations, while in patients with severe asthma these factors were age of asthma onset and frequent asthma exacerbations. Kang et al (117) found that annual incidence of asthma exacerbation and steroid use and emergency room visits due to asthma exacerbation were higher in patients with both asthma and bronchiectasis than in those with asthma alone, although the fact that the study population comprised mostly mild to moderate asthma patients may have influenced the results. Risk factors associated with bronchiectasis were also determined by Padilla-Galo et al in a moderate to severe asthma cohort using a NOPES score, including FeNO, pneumonia, expectoration and asthma severity, which were found to be independently associated with bronchiectasis. (83) In a cohort of severe asthma patients Clement et al (85) showed that older age (>40yrs) (OR: 8.3; 95% CI: 1.7– 41.2) and chronic airflow obstruction (OR: 5.4; 95% CI: 1.9 –15.3) were associated with bronchiectasis.

This study has the limitation that, although it aims to evaluate the impact of bronchiectasis on asthma, we cannot clearly determine which pathology comes first because clinical symptoms in one disease can also mask the other. However, all patients in our study initially consulted for asthma.

In conclusion, our results suggest that, in severe asthma patients, the presence of bronchiectasis is associated with age of asthma onset and the number of exacerbations. Our findings draw attention to the impact of bronchiectasis in asthma patients and should encourage clinical professionals to improve early detection and interventions for bronchiectasis in severe asthma patients, in order to improve patients' quality of life and reduce the economic burden of this disease. Further studies are needed to confirm our results and to focus on the underlying mechanisms via a study of the proinflammatory cytokines involved in this complex entity, and thus to be able to offer immunophenotype-based precision treatment.

5.2. Discussion of study 2

In this study, we evaluate the inflammatory phenotypes and the expression of proinflammatory and remodelling cytokines in asthma patients with and without bronchiectasis. The study demonstrates that the inflammatory phenotype of patients does not differ depending on the presence or absence of bronchiectasis. Moreover, to the best of our knowledge, the present study is the first to illustrate an airway remodelling activation with increased levels of transforming growth factor beta1 (TGFβ1) and vascular endothelial growth factor (VEGF) cytokines in asthma patients with bronchiectasis.

In the present study, no differences were found in the inflammatory phenotype of severe asthma patients with and without bronchiectasis. However, there was a neutrophil-predominant inflammatory phenotype in both cohorts, and almost 80% of subjects presented granulocytic inflammation (eosinophilic, neutrophilic or mixed). Indeed, both eosinophilic and neutrophilic inflammation represent a common subset of severe asthma. (131–133). In the Severe Asthma Research Program (SARP) study, Moore et al (134) found that severe asthma patients expressed high eosinophils and neutrophils in the bronchoalveolar lavage (BAL) fluid, and neutrophilic and mixed granulocytic inflammation were associated with more severe asthma phenotype. Similarly, in the U-BIOPRED hierarchical clustering study (135) the authors identified three clusters in which cluster 2 was characterized by the highest neutrophils, cluster 1 comprised mainly subjects with eosinophilic inflammation mediated by IL-13/Th2 signature and ILC2, and cluster 3 consisted mostly of patients with paucigranulocytic inflammation with a high expression of mitochondrial oxidative stress (OXPHOS) and ageing gene signatures. In any case, eosinophilic, neutrophilic or mixed granulocytic inflammation were clinically associated with a great degree of airway obstruction. (135) Studies comparing sputum inflammation in patients with asthma and bronchiectasis are scarce. Simpson et al (136) examined sputum toll-like receptor (TLR) protein expression in neutrophilic asthma and a small cohort of bronchiectasis patients as a positive reference and found that both patients with neutrophilic asthma and patients with bronchiectasis showed increased expression of the receptors TLR2 and surfactant protein A, reflecting that activation of the innate immune inflammation pattern is commonly shared in these two entities. Moreover, in patients with bronchiectasis, increased levels of sputum proteolytic enzymes such as neutrophil elastase and matrix metalloproteinase have been observed. (94) Nevertheless, although bronchiectasis is usually associated with neutrophilic inflammation, (79,137) when asthma is also present, the inflammation may also be eosinophilic or mixed (neutrophilic–eosinophilic). (138) In this context, Tsikrika et al (100) demonstrated that the presence of

significant neutrophilic inflammation is mainly related to greater bronchial destruction in HRCT and lower bronchodilator reversibility, while the presence of a high percentage of sputum eosinophils is characterized by a greater bronchodilator reversibility. In our study, no significant correlations were found between sputum eosinophils, neutrophils and parameters related to the radiological features of bronchiectasis.

Vascular endothelial growth factor and TGFβ1 are both considered essential in the airway remodelling process. Transforming growth factor beta1 is a potent profibrogenic cytokine inducing the transformation of pulmonary fibroblasts to myofibroblasts which contribute to subepithelial fibrosis by loading and accumulating high levels of collagen. (139) A role of TGFβ1 in the proliferation of smooth muscle has also been reported. (44,140,141) The increase in TGFβ1 in our cohort of severe asthma patients with bronchiectasis may represent a greater degree of asthma severity. In fact, TGFβ1 is a major mediator involved in pro-inflammatory responses and fibrotic tissue remodelling within the asthmatic lung and its role was highlighted in severe eosinophilic asthma in comparison with mild to moderate and healthy control subjects. (142) Similarly, VEGF is a potent stimulator of vascular angiogenesis promoting the development of bronchial microvasculature in asthma. (141,143,144,145). Increased expression of VEGF was found correlated with a higher degree of airway narrowing and airway vascular permeability. In this context, the bronchial wall thickening in bronchiectasis may be the consequence of a complex pro-inflammatory and inflammatory participation with the involvement of VEGF. Moreover, VEGF plays an important role in Th2 inflammation-inducing eosinophilic inflammation, mucous metaplasia, subepithelial fibrosis, myocyte hyperplasia, dendritic cell activation, and airway hyperresponsiveness via IL-13-dependent and independent mechanisms. (146). Overall, the increase in these two cytokines in our study may reflect airway remodelling in, severe asthma with bronchiectasis (Figure 5.1).

Moreover, we found a decrease in the role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the sputum of severe asthma patients with bronchiectasis. Granulocyte-macrophage colony-stimulating factor (also known as CSF2) is a multifunctional inflammatory mediator. Together with IL5, GM-CSF partially modulates the Th2 pathway, promoting the accumulation and survival of eosinophils in allergic inflammation of asthma subjects. (147) The role of GM-CSF in neutrophils, including its priming, activation and survival has also been reported. (148) Neutrophilic inflammation is widely recognized in bronchiectasis. Neutrophils as a primary component of the innate immune cells react immediately against airway infection by swallowing invaders. In an *in vitro* study, Ruchaud et al (149) suggested that GM-CSF significantly improves the blood

neutrophils' phagocytic capacity in patients with idiopathic bronchiectasis. Furthermore, a study in mouse models confirmed that GM-CSF maintains the normal pulmonary physiology and resistance to local infection, playing an essential role in host defence. (150) Granulocyte-macrophage colony-stimulating factor may also have a critical role in mediating the Th2 allergic inflammation pathway. (151,152) In the present study, the decrease of this cytokine may have led to impairment of the neutrophil function in asthma patients with bronchiectasis. Further study of these CSF subtypes in well-specified asthma phenotypes is now required to strengthen our understanding of the immunological mechanism (Figure 5.1).

Interferon-gamma (IFN-γ) is a marker of Th1 pathway inflammation. Increased expression of this cytokine has been demonstrated in severe asthma in comparison to mild and moderate asthma. (150) Duvall et al (154) found that severe asthma patients had high ratios of Th1-enriched CD4+ T cells to natural killer (NK) cells in bronchoalveolar lavage liquid compared with non-severe asthma patients and heathy controls. Moreover, studies conducted in children have shown severe asthma to be associated with higher production of IFN-γ in bronchoalveolar lavage cells. (155) In house dust mite-sensitized mouse models of asthma, IFN-γ seems to contribute to the epithelial disruption if the T cell myeloid IL-10 axis is blocked. (156) Moreover, IFN-γ seems to suppress the function of secretory leukocyte protease inhibitor (SLPI), a protein that exhibits antimicrobial activities and is thought to play a critical role in mucosal defence. (157) Secretory leukocyte protease inhibitor was inversely correlated with IFN-γ expression. (158) The balance between proteolytic enzymes (proteases, including elastases) and their inhibitors (proteinase inhibitors) also consists of an underlying mechanism of bronchiectasis. (79,159) Overexpression of these cytokines may reflect a major Th1 pathway inflammation with an increased risk of altering the normal physiological defence mechanism and proteolytic effect. (Figure 5.1)

This study has two main limitations that should be mentioned. Firstly, with regard to its cross-sectional design, we stress that all subjects were taking standard asthma medication including inhaled glucocorticosteroid therapy. This may have affected the sputum differential cell percentage, since glucocorticosteroids can promote the apoptosis of eosinophils, although they seem to be insensitive to neutrophils in severe asthma. (160) Secondly, cytokines reflecting the Th1 inflammatory pathway (i.e., IL5 and IL13) were undetectable in our pilot study, and so their roles and possible interactions with the non-Th2 inflammation may be underestimated.

In conclusion, the present study shows that there are no differences in the inflammatory phenotype of asthma patients with and without bronchiectasis. It is also the first report of the role of airway remodelling in asthma patients with bronchiectasis. The results highlight the potential significance of non-invasive methods of assessing airway inflammation. It remains to be elucidated whether these tools will help in the future to define different disease phenotypes and whether they can provide clinically relevant information regarding disease prognosis and response to treatment.

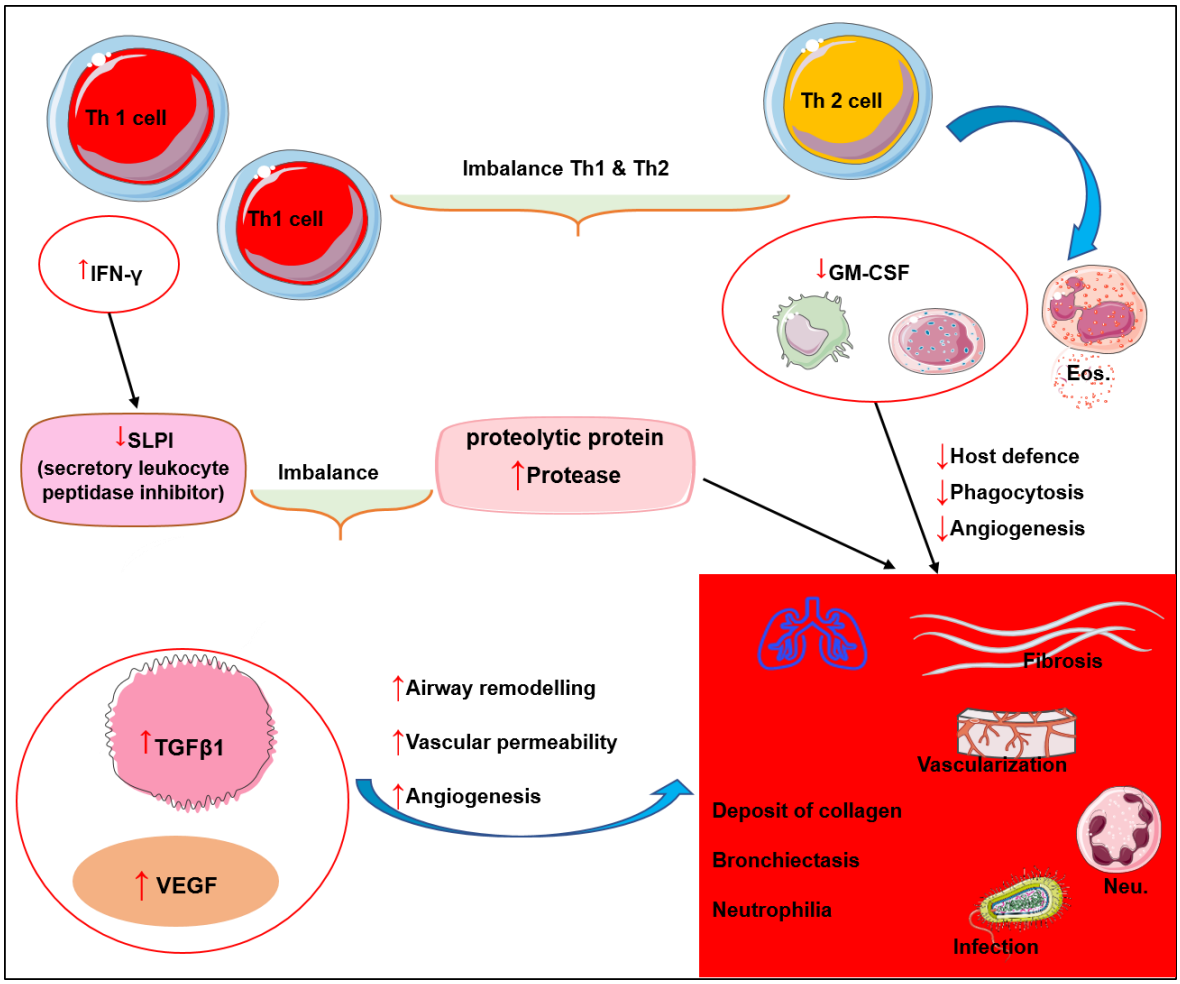

Figure 5.1. A proposed diagram of the underlying mechanism in the development of bronchiectasis in severe asthma patients. IFNγ is a potent mediator of the Th1 pathway. GM-CSF is involved in the activation and survival of eosinophils in the Th2 pathway. Imbalance of Th2 and Th2 pathway makes the inflammatory phenotype move towards neutrophilic inflammation. Increasing IFNγ results in indirect activation of proteolytic proteins such as protease, thus facilitating the destruction of the airway structure. TGFβ1 and VEGF are potent airway remodelling cytokines. Eos, eosinophils; neu, neutrophils.

CONCLUSIONS

6. CONCLUSIONS

The following conclusions can be drawn from the two studies.

1. The prevalence of bronchiectasis is quite high in asthma patients, especially in those with severe asthma.

2. In severe asthma patients, age of asthma onset and exacerbations are independent factors associated with the occurrence of bronchiectasis. In these patients, the presence of bronchiectasis may aggravate the clinical expression of asthma and provoke more asthma exacerbations and emergency visits, thus challenging asthma management.

3. The type of inflammation in asthma patients does not vary depending on the presence or absence of bronchiectasis.

4. Airway remodelling activation with increased levels of transforming growth factor beta1 (TGFβ1) and vascular endothelial growth factor (VEGF) cytokines in asthma patients with bronchiectasis is observed.

FUTURE LINES OF RESEARCH

7. FUTURE LINES OF RESEARCH

The present results show the importance of the impact of bronchiectasis in asthma patients. In these patients, early detection and interventions for bronchiectasis, especially in severe asthma patients, would improve patients' quality of life and reduce the economic burden of this disease. For this reason, future studies should be directed to focus on the underlying mechanisms involved in this complex entity, and thus to be able to offer immunophenotype-based precision treatment. Moreover, the thesis highlights the potential significance of non-invasive methods of assessing airway inflammation. Future studies should be directed to elucidate whether these tools will help in the future to define different disease phenotypes and whether they can provide clinically relevant information regarding disease prognosis and response to treatment.

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ANNEXES

9.1. ANNEX I. QUESTIONNAIRES

Asma Control Test (para mayores de 12 años)

Nombre ..... Fecha .....

1. Durante las últimas 4 semanas, ¿con qué frecuencia le impidió el asma llevar a cabo sus actividades en el trabajo, la escuela o el hogar?
   1. Siempre
   2. Casi siempre
   3. Algunas veces
   4. Pocas veces
   5. Nunca

2. Durante las últimas 4 semanas, ¿con qué frecuencia ha sentido que le faltaba el aire?
   1. Más de una al día
   2. Una vez al día
   3. De 3 a 6 veces por semana
   4. Una o dos veces por semana
   5. Nunca

3. Durante las últimas 4 semanas, ¿con qué frecuencia le despertaron por la noche o más temprano de lo habitual por la mañana los síntomas de asma (sibilancias/pitos, tos, falta de aire, opresión o dolor en el pecho)?
   1. Cuatro noches o más por semana
   2. De 2 a 3 noches por semana
   3. Una vez por semana
   4. Una o dos veces
   5. Nunca

4. Durante las últimas 4 semanas, ¿con qué frecuencia ha utilizado su inhalador de rescate (ejemplo, salbutamol, Ventolín)?
   1. 3 veces o más al día
   2. 1ó 2 veces al día
   3. 2ó 3 veces por semana
   4. Una vez por semana o menos
   5. Nunca

5. ¿Cómo calificaría el control de su asma durante las últimas 4 semanas?
   1. Nada controlada
   2. Mal controlada
   3. Algo controlada
   4. Bien controlada
   5. Totalmente controlada

Resultado: 25pts: máximo control; De 20 a 25: Buen control del asma; <20: Asma no controlada.

Hospital Ansiedad y Depresión Escala (HADs)

Nombre ..... Fecha .....

Este cuestionario ha sido diseñado para ayudarnos a saber cómo se siente usted. Lea cada frase y marque la respuesta que más se ajusta a cómo se sintió durante la semana pasada. No piense mucho las respuestas. Lo más seguro es que si responde deprisa sus respuestas se ajustarán mucho más a cómo realmente se sintió.

D	A		D	A	
		Me siento tenso o nervioso:			**Me siento como si cada día estuviera más lento:**
	3	Todos los días	3		Por lo general, en todo momento
	2	Muchas veces	2		Muy a menudo
	1	A veces	1		A veces
	0	Nunca	0		Nunca
		Todavía disfruto con lo que antes me gustaba:			**Tengo una sensación extraña, como si tuviera mariposas en el estómago**
0		Como siempre		0	Nunca
1		No lo bastante		1	En ciertas ocasiones
2		Sólo un poco		2	Con bastante frecuencia
3		Nada		3	Muy a menudo
		Tengo una sensación de miedo, como si algo terrible me fuera a suceder			**He perdido mi interés en mi aspecto personal:**
	3	Definitivamente y es muy fuerte	3		totalmente
	2	Sí, pero no es muy fuerte	2		No me preocupo tanto como debiera
	1	Un poco, pero no me preocupo	1		Podría tener un poco más de cuidado
	0	Nada	0		Me preocupo al igual que siempre
		Puedo reírme y ver el lado divertido de las cosas:			**Me siento inquieto, como si no pudiera para de moverme:**
0		Al igual que siempre lo hice		3	Mucho
1		No tanto ahora		2	Bastante
2		Casi nunca		1	No mucho
3		Nunca		0	Nada
		Tengo mi mente llena de preocupaciones:			**Me siento optimista respecto al futuro**
	3	La mayoría de las veces	0		Igual que siempre
	2	Con bastante frecuencia	1		Menos de lo que acostumbraba
	1	A veces, aunque no muy a menudo	2		Mucho menos de lo que acostumbraba
	0	Sólo en ocasiones	3		Nada
		Me siento alegre:			**Me asaltan sentimientos repentinos de pánico:**
3		Nunca		3	Muy frecuentemente
2		No muy a menudo		2	Bastante a menudo
1		A veces		1	No muy a menudo
0		Casi siempre		0	Rara vez
		Puedo estar sentado confortablemente y sentirme relajado:			**Me divierto con un buen libro, la radio, o un programa de televisión:**
	0	Siempre	0		A menudo
	1	Por lo general	1		A veces
	2	No muy a menudo	2		No muy a menudo
	3	Nunca	3		Rara vez

Por favor, compruebe que ha respondido todas las preguntas

Puntuación: 0-7 = Normal; 8-10 = límite anormal (caso límite); 11-21 = Anormal (caso)

Puntuación total: Depresión (D) _____ Ansiedad (A) _____

MiniAQLQ: CUESTIONARIO DE CALIDAD DE VIDA EN PACIENTES CON ASMA VERSIÓN REDUCIDA

Nombre ..... Fecha.....

Le rogamos responda **a todas** las preguntas señalando con un círculo la respuesta que mejor describa cómo se ha encontrado **durante las dos últimas semanas, debido al asma.**

En general, ¿con qué frecuencia durante las 2 últimas semanas:								
		siempre	casi siempre	gran parte del tiempo	parte del tiempo	poco tiempo	casi nunca	nunca
1	Notó que le faltaba el ¿Aire debido al asma?	1	2	3	4	5	6	7
2	¿Sintió que le molestaba el polvo, o tuvo que evitar un lugar debido al polvo?	1	2	3	4	5	6	7
3	¿Se sintió frustrado o irritado debido al asma?	1	2	3	4	5	6	7
4	¿Sintió molestias debido a la tos?	1	2	3	4	5	6	7
5	¿Tuvo miedo de no tener a mano su medicación para el asma?	1	2	3	4	5	6	7
6	¿Notó una sensación de ahogo u opresión en el pecho?	1	2	3	4	5	6	7
7	Sintió que le molestaba el humo del tabaco, o tuvo que evitar un lugar debido al humo del tabaco?	1	2	3	4	5	6	7
8	¿Tuvo dificultades para dormir bien por la noche debido al asma?	1	2	3	4	5	6	7
9	¿Se sintió preocupado por tener asma?	1	2	3	4	5	6	7

10	¿Sintió silbidos o pitos en el pecho?	1	2	3	4	5	6	7
11	¿Sintió que le molestaba o tuvo que salir de casa debido al tiempo o a la contaminación atmosférica?	1	2	3	4	5	6	7

¿Hasta qué punto el asma le ha limitado para hacer estas actividades durante las 2 últimas semanas?								
		Total-mente limitado	Estremada-mente limitado	muy limitado	Moderada-mente limitado	algo limitado	poco limitado	nada limitado
12	Esfuerzos intensos (como darse prisa, hacer ejercicio, subir escaleras corriendo, hacer deporte)	1	2	3	4	5	6	7
13	Esfuerzos moderados (como caminar, hacer las tareas del hogar, trabajar en el jardín o en el huerto, hacer la compra, subir escaleras sin correr)	1	2	3	4	5	6	7
14	Actividades sociales (como hablar, jugar con niños/animales domésticos, visitar a amigos/familiares)	1	2	3	4	5	6	7
15	Actividades relacionadas con su trabajo (tareas que tiene que hacer en su trabajo*)	1	2	3	4	5	6	7

* Si no está trabajando, responda a esta pregunta pensando en las tareas que tiene que hacer la mayoría de los días.

ESCALA MODIFICADA DE DISNEA (mMRC)

Nombre ……. Fecha …….

ESCALA MODIFICADA DE DISNEA (mMRC)	
GRADO	ACTIVIDAD
0	Ausencia de disnea al realizar ejercicio intenso
1	Disnea al andar deprisa en llano, o al andar subiendo una pendiente poco pronunciada.
2	La disnea le produce una incapacidad de mantener el paso de otras personas de la misma edad caminando en llano o tener que parar para descansar al andar en llano a su propio paso.
3	La disnea hace que tenga que parar a descansar al andar unos 100 metros o pocos minutos después de andar en llano.
4	La disnea impide al paciente salir de casa o aparece con actividades como vestirse o desvestirse.

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