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Nicolas MIGUERES

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PHENOTYPES OF OCCUPATIONAL ASTHMA

Insights from E-PHOCAS and EGEA cohorts

PHENOTYPES DE L'ASTHME PROFESSIONNEL

Dans le cadre des cohortes E-PHOCAS et EGEA

THÈSE dirigée par :

GODET Julien	PU-PH, Université de Strasbourg
SIROUX Valérie	DR Inserm, Université Grenoble Alpes

RAPPORTEURS :

GARCIA AYMERICH Judith	Research Professor, Barcelona Institute for Global Health
GUILLEMINAULT Laurent	PU-PH, Université Paul Sabatier

AUTRES MEMBRES DU JURY :

HUMBERT Marc	PU-PH Université Paris Saclay
GONZALEZ Maria	PU-PH, Université de Strasbourg

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- **Migueres N**, Vandenplas O, Walusiak-Skorupa J, Wiszniewska M, Munoz X, Romero-Mesones C, Suojalehto H, Lindstrom I, van Kampen V, Merget R, Mason P, Maestrelli P, Sastre J, Rifflart C, Godet J, de Blay F. Sputum Inflammatory Patterns are Associated with Distinct Clinical Characteristics in Subjects with Occupational Asthma Independently from Causal Agent. *Journal of Investigational Allergology and Clinical Immunology* 2022;
- **Migueres N**, Vandenplas O, Walusiak-Skorupa J, Munoz X, Suojalehto H, Van Kampen V, Mason P, Quirce S, de Blay F, European network for the PHenotyping of OCcupational ASthma (E-PHOCAS). Work-related dysphonia in subjects with occupational asthma is associated with neutrophilic airway inflammation. *Clinical & Translational All* 2023; 13: e12218

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Oral Communication

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Posters:

- **Migueres N**, O. Vandenplas, H. Suojalehto, J. Walusiak-Skorupa X. Munoz, J. Sastre, R. Merget, G. Moscato, S. Quirce, N. Meyer, J. Godet, F. De Blay Phenotypes of occupational asthma defined with induced sputum after specific inhalation challenge *ERS international virtual congress 6 September 2021*
- **Migueres N**, Vandenplas O, Walusiak-Skorupa J, Munoz X, Suojalehto H, Van Kampen V, Mason P, Quirce S, de Blay F, European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) Work-related dysphonia in subjects with sensitizer-induced occupational asthma is associated with neutrophilic inflammation *ERS international congress, Barcelona 4 September 2022*
- **Migueres N**,Dumas O, Boudier A, Bousquet J, Varraso R, Nadif R, de Blay F, Le Moual N, Siroux V , Allergen-specific IgE sensitization and occupational exposure to irritants *ERS international congress, Vienna 9 September 2024*

Invited conference

- **Migueres N**. Vandenplas O, Walusiak-Skorupa J, Munoz X, Suojalehto H, Van Kampen V, Mason P, Quirce S, de Blay F, European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) *European Academy of Allergy & Clinical Immunology Congress Hamburg 09/06/2023:* Work-related asthma, from phenotypes to endotypes: when did we start and where are we?

[Conference concerning the concept of endotype in occupational asthma]

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[A conference on the prelimary result of E-PHOCAS study on inflammatory phenotype in occupational asthma]

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Publications and communications outside the thesis work

Published article:

- **Migueres N**, de Blay F, Braun JJ. Complex rhinobronchial dystrophy and immunodeficiency: Chance association or exceptional congenital syndrome? *European Annals of Otorhinolaryngology, Head and Neck Diseases* 2020; 137: 135–137.

[A case report on a congenital malformation with congenital anosmia, severe atrophic rhinitis, common variable immunodeficiency and tracheobronchial diverticula.] Contribution: redaction of the first draft and review, validation.

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[A comparative study which show that dysphonia is prevalent in asthmatic women, and is not explained by organic lesions after laryngeal examination. Laryngeal dysfunction was observed in a majority of patients.] Contribution: redaction of the first draft, review, validation.

- Marcot C, **Migueres N**, Ott M, Khayath N, De Blay F. Polluants allergéniques et chimiques de l'environnement intérieur et asthme : caractérisation, évaluation et éviction. *Revue des Maladies Respiratoires* 2023; 40: 630–645.

[A review of the literature about the characterization on indoor pollution and the proof of efficacy of global eviction in asthmatic patients]

- Melscoet L, Khayath N**, Migueres N**, Goltzene M-A, Meyer N, de Blay F. Severe non-atopic asthma: omalizumab can reduce severe asthma exacerbations. *Journal of Asthma* 2023; 60: 881–889.

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- Van Kampen V, **Migueres N**, Doyen V, Deckert A, de Blay F, Vandenplas O, Merget R. Phenotyping occupational asthma caused by platinum salts compared with other low–molecular weight agents. *The Journal of Allergy and Clinical Immunology: In Practice* 2023; 11: 2929-2932.e2.

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- Mwanga HH, Dumas O, **Migueres N**, Le Moual N, Jeebhay MF. Airway diseases related to the use of cleaning agents in occupational settings. *J Allergy Clin ImmunolPract* 2024;: S2213-2198(24)00214-9.

[A review of the recent literature about airway disease resulting or exacerbated by cleaning agents in occupational setting] Contribution: redaction of one section,review, validation

- Doyen V, **Migueres N**, Frère A, Walusiak-Skorupa J, Wiszniewska M, Suojalehto H, et al. Diagnostic Accuracy of Specific IgE Against Wheat and Rye in Flour-Induced Occupational Asthma. J Allergy Clin Immunol Pract. mai 2024;S2213219824005282.

[An original article showing the accuracy of spectifc IgE againt wheat and rye in flour induced asthma. High level of specific IgE was associated with a high predictive positive value of occupational asthma.] Contribution: Analysis of data, review, validation

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Communication

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- **Migueres N,** Poirot A, Zhang N, Bachert C, de Blay F. Omalizumab effectiveness is independent of Staphylococcal Enterotoxin sensitization. *ERS international virtual congress September 2021*

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[Conference about occupational allergic risk, and risk factor]

- **Migueres N**, Vandenplas O, Walusiak-Skorupa J, Munoz X, Suojalehto H, Van Kampen V, Mason P, Quirce S, de Blay F, European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) *Congrès Français d'Allergologie Paris 19/04/2024:* Les pathologies irritatives : que faut-il comprendre ?

[Conference about irritative respiratory disease, from RADS to chronic refractory cough]

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List of abbreviations

AA : Arachidonic acid

AERD: Aspirin exacerbated respiratory disease

AIT: Allergen specific immunotherapy

CAS: Chemical Abstracts Service

CI: confidence interval

COX-1: cyclooxygenase-1

CRSwNP : chronic rhinosinusitis with nasal polyps

CRTH2: Type 2 helper cells

DALYs: Disability adjusted life years

EIB: Exercise induced bronchoconstriction

E-PHOCAS: European network on Phenotyping of Occupational Asthma

FeNO: Fractional exhaled nitric oxide

FEV1: Forced Expiratory Volume in 1 second

FVC: Forced vital capacity

GINA: Global Initiative for Asthma

HMW: High-molecular-weight

ICS: Inhaled Conrticosteroid

IgE: Immunoglobulin E

OIE: Occupational irritant exposure

ILC2: Innate lymphoid cell type 2

ILO: Inducible laryngeal obstruction

IL: interleukin

iNOS: Inducible nitric oxide synthase

IQR: Interquartile range

LABA: Long-acting beta-2-agonist

LTs: Leukotrienes

LMW: Low-molecular-weight agents

N-ERD: Nonsteroidal anti-inflammatory drug- exacerbated respiratory disease

NSAID: Nonsteroidal anti-inflammatory drug

NSBH: Nonspecific bronchial hyperresponsiveness

OA: Occupational asthma

ORMDL3: Orosomucoid-like 3

PEF: Peak expiratory flow

PGs: Prostaglandins

QSAR : Quantitative structure activity relationship

RNV3P: Réseau National de Vigilance et de Prévention des Pathologies Professionnelles

SABA: Short-acting beta-2 agonist

sIgE: allergen-specific immunoglobulin E

SLIT: Sublingual immunotherapy

TENOR: The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens

Th2: Type 2 helper

TRPA1: Transient receptor potential cation channel subfamily A member 1

TSLP: thymic stromal lymphopoietin

TXs: Thromboxanes

WEA: work -exacerbated asthma

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Introduction

Classification shapes medicine and guides its practice [7]. One of the main aim of classification according to Richardson is to reduce a disordly mass to an ordely whole [7,8]. Classificatory medicine emerged during XVII century with the work of Thomas Sydenham intending to set up a nosologic classification of diseases in the same way than the botanist Linnaeus realized the taxonomy of every living being in *Systema Naturae* (1735), listing the characteristics of diseases by symptoms. The school of thought of classificatory medicine failed to develop a standardized classification of diseases, provoking Flaubert astonishment: "Sauvages had admitted 1,800 species. Cullen less than 600. Sagar 2 thousand 5 cents."[9]. He developed his criticism in his last unfinished book, *Bouvard et Pécuchet* : « On les compte par milliers, et la classification linnéenne est bien commode, avec ses genres et ses espèces ; mais comment établir les espèces ? » [10].

What are the criterions for an effective classification in medicine? We could expect it to be able to gather individuals sharing common traits, common disease processes, to predict the evolution of the disease and to predict treatments' responses. Modern medicine enables a far higher level of biological and clinical precision and integration than in XVII century. Researchers are now aiming for "precision medicine" which has been defined as "treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations"[11,12]. As an example, the traditional classification of lung cancer based on anatomic and histologic criteria has been augmented by molecular genetic markers as EGFR testing [11].

Asthma is defined by the history of respiratory symptoms, such as wheeze, shortness of breath, chest tightness and cough, that vary over time and in intensity, together with variable expiratory airflow limitation [13]. Asthma is one of the most frequent chronic respiratory diseases. Globally, the total number of patients with asthma was estimated to 262 million [95% UI: 224–

309 million] in 2019 with large geographic variability. Estimations suggest a global prevalence of asthma symptoms of around 10 % in children and adolescents and 6-7% in adults [14–16]. In France, the prevalence of current adult asthma is estimated to be 5.8 % (5.1 % in men, 6.4 % in women) [17,18]. The worldwide burden of asthma is massive, it accounted for a total of 0.460 million [0.367–0.559] deaths in 2019, resulting in an age-standardized mortality rate of 5.8 per 100 000 population [17]. The impact of asthma shows a large geographical heterogeneity: the prevalence tends to be higher in countries with higher socio-demographic index but countries with lower socio-demographic index show higher mortality and disability adjusted life years (DALYs) [17]. Occupational asthma, whose definition is an asthma which could not be explained by a cause exterior to workplace, is estimated to account for 16% (95% CI, 10–22%) of all asthma cases [19].

The single term “asthma” may lie umpteen meanings. First, the meaning of the term “asthma” has varied during history. The etymology of asthma come from ancient Greek, ἆσθμα (âsthma), first used by Homer in the *Iliad* (800 BCE) speaking about a warrior who died at the end of a furious battle with "asthma and perspiration", referring for a short-drawn breath [20]. The earliest text where the word appears as a medical term is the *Corpus Hippocraticum*, text in which it is difficult to determine whether “asthma” refers to an autonomous clinical entity or simply a symptom as it is used indiscriminately with the term dyspnea, tachypnea and orthopnea [20]. Secondly, even nowadays asthma is described as a heterogeneous entity, even a syndrome, regrouping different underlying disease processes because of the diversity of clinical presentations [21–23]. The Lancet published a plea to abandon asthma as a disease concept, hypothesizing that asthma could be compared to fever, which was regarded as a disease until the 19th century [24]. That’s why attempts have been made to classify asthmatic patients in different phenotypes, a phenotype being the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Classification of asthma is dynamic, and has changed considerably during the last decades [21,25–28]. Clinical differences, such as the type of trigger or the age of onset, have, for a long time, been the main criterions to define phenotypes. Then, the characterization of the type of airway inflammation has augmented our understanding of asthma heterogeneity and the variability of the treatments' responses.

Occupational asthma has been considered as a model for the understanding of asthma in general because it represents an experimental situation in which subjects can be examined before, during, and after stopping exposure [29]. Nevertheless the classification of occupational asthma has hardly evolved, based on empirical constatations in the 80's [30,31]. More investigations are needed to determine whether phenotyping of occupational asthma according to trigger factor, clinical presentation and inflammatory pattern will facilitate a more comprehensive understanding of the heterogeneity of this condition. This thesis aimed to identify phenotypes of occupational asthma based on the offending agents, the clinical presentation and inflammatory profiles.

The first part of the thesis will introduce well-defined phenotypes of asthma and describe how trigger factor, clinical presentations and inflammation profiles enable to distinguish distinct groups of patients. The second part will focus on the current classification of occupational asthma and underline its limits.

First Part:
Asthma phenotypes

The first modern classification of asthma was proposed by Rackemann in 1947 [32]. It was based on the age of onset and trigger of symptoms. Extrinsec asthma begins before the age of 30 and is driven by allergies. Intrinsec asthma begins after the age of 40 and is associated more frequently with nasal polyps. This classification have been rejected because of the growing interest for eosinophil inflammation, which has been observed as predicting response to corticosteroid therapy [33] and the lack of immunologic difference between intrinsic and extrinsic asthma [34–36]. In order to disentangle the complexity of asthma, supervised and unsupervised method have been performed to identify asthma phenotypes [28]. This section will begin by outlining the phenotypes based on disease triggers and clinical presentation. This will be followed by an examination of the phenotypes related to inflammation, and finally, the results of the unsupervised classification analysis will be presented.

1.1 Trigger related phenotypes

Asthma, as a paroxysmal disease, has always raised questions about triggers of the disease. Hippocrates (460–370 BCE), described panting caused by occupational exposures as in metal workers, tailors, horsemen, farmhands and fishermen [37]. In the first modern description of asthma in Sir John Floyer's *A treatise of the Asthma* (1698), he proposed different triggers such as the barometric pressure, temperature, diet, exercise, sleep [38]. The first classification of asthma by Rackemann in 1947 also depended of triggers differentiating extrinsic asthma for which "the trouble is outside the patient" as allergy, and intrinsic asthma for which the cause is "inside the body"[32].

1.1.1 Allergic asthma

Definition of type 1 hypersensitivity and atopic march

Allergic asthma is defined as IgE mediated asthma, which corresponds to Type 1 hypersensitivity, that can also occur in patients with atopic dermatitis, acute urticaria, food venum and drug allergy [39]. The individual presents an IgE sensitization to an aeroallergen, and exposure to this allergen brings about asthma symptoms. Type 1 response includes two phases (Figure.1) [39]. The sensitization phase depends on T2 cell signals which regulate allergen-specific immunoglobulin E (sIgE) production. Upon initial exposure to an allergen, the allergen is internalized by antigen-presenting cells, including dendritic cells, lymphocyte B cells and macrophages. They process and present the allergen peptides on their surface, linked to major histocompatibility complex class II molecule to naïve T cells. Dendritic cells, through the expression of surface molecules, the secretion of metabolites, and the release of cytokines, facilitate the activation and differentiation of naïve T cells into Th2 cells. Innate lymphoid cells type 2 (ILC2) are activated by cytokines released by epithelial cells (called alarmins) such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). Upon activation, they produce a large amount of type 2 cytokines, including IL-5, IL-9 and IL-13 further supporting the T2 response. T helper cells assist B cells in maturing and producing high-affinity IgE. The effector phase occurs upon subsequent exposure to the same allergen. The allergen crosslinks IgE bound to mast cells and basophils, triggering degranulation and releasing mediators that induce symptoms such as bronchial muscle contraction and increased mucus secretion.

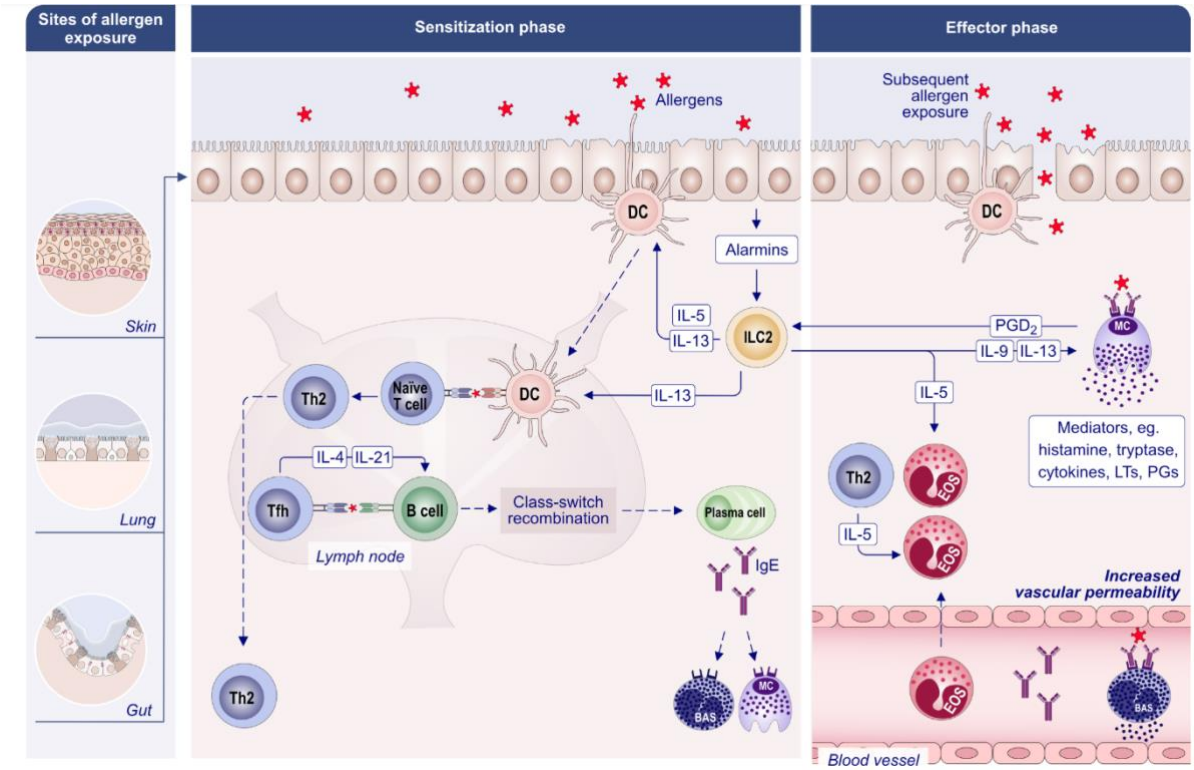

Figure 1 :Mechanisms of type 1 hypersensitivity, from [39]

Atopy is the tendency to produce an exaggerated IgE immune response to otherwise harmless substances in the environment [40]. The atopic march has been defined as the natural history of atopic manifestations characterized by a typical sequence of progression of clinical signs of atopic disease, with some signs becoming more prominent while others subside [41]. Predisposed subjects will be more likely to develop first atopic dermatitis and food allergy, then allergic rhinitis and then asthma. It is estimated that approximately one-third of individuals with atopic dermatitis will develop asthma, while two-thirds will develop allergic rhinitis over the course of their lifetime [42,43]. For example, Rhodes et al described the evolution of a birth cohort selected on the basis that one or both parents had a history of asthma and/or hayfever, and followed them for 22 years [44,45]. The prevalence of atopic dermatitis peaked at 20% of children by 1 year of age and declined to approximately 5% of patients at the end of the study.

Meanwhile, the prevalence of allergic rhinitis slowly increased over time from 3% to 15%. Concerning asthma (defined as bronchial hyperresponsivness and wheezing), the prevalence was to 24% at 11 years old and stay the same at 25% at 22 years old. In addition, sensitization to allergen by skin prick test to 1 of 6 allergens (Dermatophagoides pteronyssinus, mixed grass, dog, cat, egg, and milk) increased to a peak of 36% at 22 years of age.

One of the main risk factors to develop allergic diseases is to have close relative with an allergic disease. A child with one allergic parent has a 25% risk of allergy whereas a child with two allergic parents has a 50% risk of allergy. Genetic studies have uncovered multiple genes with a possible role in the development of atopic diseases [46,47]. Their clinical expressions results from complex interaction between these genes and environmental factors [47]. Skin barrier function have a great role in the process of developing IgE sensitization. Loss of function genetic variant in the gene encoding filaggrin, a key protein of the epidermidis and formation of skin barrier, show a significant association with atopic dermatitis and allergic asthma [48,49].

Clinical presentation

Allergic asthma is the most common asthma phenotype [50,51]. It is estimated that allergic mechanisms are implicated in 80% of childhood asthma [52,53] and in about 40-50% of adult form [53,54]. The prevalence of allergic asthma is higher in males during childhood, with a more balanced sex ratio observed after puberty [55]. In the latter, seasonal exacerbation in function of the profile of sensitization is observed [56]. Allergic asthma develops mainly during childhood and have a longer duration of disease than non-allergic asthma [56,57]. It is often associated with eczema and allergic rhinitis [41,44]. Up to 60 % of patients with childhood wheezing are expected to experience remission of their disease during adulthood [58–60]. Allergic comorbidity as allergic rhinitis and atopic dermatitis, parental history of asthma as well as polysensitization are at a risks of a persistence of childhood asthma during adulthood

[16,61,62]. The Asthma Predictive index, developed to determine the likelihood of a child experiencing wheezing to experience subsequent asthma, includes allergic components. Conversely, childhood eczema or rhinitis is not associated with non-allergic adult asthma suggesting different underling disease process between allergic and non-allergic asthma [62].

The clinical presentation of allergic asthma phenotype is heterogeneous as is IgE sensitization. Allergic multimorbidity concept state that asthma or rhinitis alone are not the same disease than association of allergic diseases (asthma, rhinitis, atopic dermatitis) [63]. Association of allergic diseases, called allergic multimorbidity, is associated with IgE polysensitization while asthma or rhinitis alone are predominantly associated with IgE monosensitization. Monosensitization has been associated with lower total and specific IgE levels and with fewer epitope recognition [64–66]. It has been shown that the number of IgE sensitization is associated positively with the number of allergic-related diseases and that the type of IgE sensitization is associated with different allergic-related phenotypes [67]. Indeed, in the EGEA study, using unsupervised cluster analysis, the IgE sensitization profile "Pollen/animal allergens" was associated with rhinitis alone, the "most prevalent house dust mite allergens" profile was association with asthma alone, and the "many allergens" profile was associated with rhinitis and asthma [68]. In addition, sensitizations to some specific allergens were identified as independent risk factor for developing asthma. In particular, house dust mite sensitization has been described as a risk factor for asthma in adult [69,70]. This effect could be explained by the fact that *Dermatophagoides pteronyssinus* allergen I, major allergen of house dust mite, provoke a proteolytic cleavage of tight junction and alter epithelial barrier[70,71].

Diagnostic biomarkers for allergic asthma:

Total IgE has been proposed as a biomarker of allergic asthma. Total IgE levels are usually higher in allergic versus nonallergic asthma [72]. Nevertheless, Burrows et al. demonstrated

that total IgE are associated with asthma independently of allergic rhinitis [73]. Indeed, elevation of total IgE is also observed in non-allergic asthmatic patients [74]. The specificity of these IgE remains elusive [70,75]. Superantigens, a class of antigen provoking an excessive activation of the immune system and production of polyclonal IgE, may contribute to this elevation [75]. *Staphylococcus aureus* enterotoxin, which is both a B cell and a T cell superantigen may contribute to the elevation of total IgE [76]. Sensitization to *Staphylococcus aureus* enterotoxin can frequently be detected in serum and has been associated with asthma and severe asthma [76,77].

Total IgE is influenced by various non atopic factors. Elevated total IgE is present in parasite infection (strongyloidiasis, ascariasis, schistosomiasis), inflammatory disorders (Kimura disease, Churg-Strauss vasculitis, Kawasaki's disease), hematologic disorders (Hodgkin's lymphoma, IgE myeloma) and primary immunodeficiency disorders (Wiskott-Aldrich syndrome, Omenn syndrome, IPEX, and atypical complete DiGeorge syndrome)[78]. Some environmental factors such as tobacco use, air pollution exposure has been observed as associated with higher levels of total IgE [79–81].

Specific IgE and skin prick test to environmental allergens inform of sensitization and are associated with allergic asthma. However, sensitization alone, may not be clinically relevant [82]. Screening of non-symptomatic individuals with specific IgE tests has a low positive predictive value for allergy, in the range of 50% for regular cut off of 0.35kU/l [82,83]. Higher titer of specific IgE and allergen component specific IgE have been shown as being more strongly associated with asthma or clinical allergic manifestations than regular thresholds (>0.35KU/L) [82,84,85].

Treatment and management

The management of allergic asthma includes multiple approaches. To begin, environmental control. As triggered by environmental allergens, eviction of allergens is a key treatment of allergic asthma. First was evaluated specific eviction of specific allergens. Gøtzsche et al. evaluated the efficacy of house dust mite control measures (physical and/or chemical methods) in HDM sensitized asthma management strategy in a meta-analysis gathering 55 studies and 3121 patients [86]. The implementation of house dust mite control measures did not result in a notable enhancement in the assessed outcomes, including medication intake, symptom score, and peak flow measurement, when compared to the control group. Doubts of the applicability of these results have been suggested because of the baseline characteristics of the patients. Van Boven et al. performed a meta-analysis on the baseline characteristic of patients included showing that mite avoidance studies were characterized by the inclusion of patients with rather mild to moderate asthma and with varying and sometimes negligible levels of allergen exposure that could have biased the results [87,88]. Global eviction via multifaced intervention, consisting of allergen eviction, reduction of exposure to tobacco smoke and irritants, has been demonstrated efficient in randomized controlled trials, in reducing the rate of exacerbations, in children with a high rate of exacerbation coming from low-income families [89,90]. More recently, a double blind randomized controlled trial showed an efficacy of house dust mite eviction by impermeable bedding on exacerbation in severe allergic asthmatic children [91]. In post-hoc analysis, the younger patients and those living in the most deprived homes beneficiated most of the measure. Indoor environment advisors intervention can be prescribed in France, in order to help improving insight of the disease and to promote global eviction [92,93].

Allergen specific immunotherapy (AIT) is an allergic tolerance-inducing treatment for allergic disease. It consists in the administration of increasing amounts of specific allergens to which the patient has type I immediate hypersensitivity. Immunotherapy has traditionally been administered subcutaneously, however, sublingual immunotherapy (SLIT) has been shown to

be a safe and effective alternative [94]. AIT aims to induce allergen-specific regulatory T cells (Treg) and their associated suppressor cytokines, including IL-10, TGF-β, and surface molecules such as CTLA-4 and PD-1 [95,96]. Collectively, these elements contribute to the establishment of a suppressive immune environment. The modulation of T- and B-cell responses, antibody isotypes, and the functional limitation of mast cells, eosinophils, and basophils collectively result in the induction of long-term allergen-specific immune tolerance [97,98]. Allergen- specific immunotherapy is the only specific and disease-modifying treatment for allergic conditions [95]. It is a therapy that has demonstrated the capacity not only to improve symptoms, reduce the need for medications, but also to induce specific tolerance beyond the duration of the treatment and to prevent the development of new allergic conditions [95,99–101]. Concerning efficacy in asthma, the level of proof is higher for subcutaneous immunotherapy than for SLIT [100,102–104]. Although AIT can be used in patients with well-controlled asthma, it is not routinely used in severe asthma because of the risk of exacerbation and systemic reaction that is increased in uncontrolled patients [101].

Omalizumab, a biologic agent, has been designed for allergic asthma. Omalizumab is a humanized monoclonal antibody that binds free IgE and inhibits the binding of IgE to the IgE high-affinity receptor (FcεRI) and CD23 which is a low affinity receptor. It reduces the expression of FcεRI in mast cells, basophils and dendritic cells, blocking the allergic response [105]. It has been approved for subjects with moderate-to severe allergic uncontrolled asthma with asthma symptoms due to exposure to a perennial aeroallergen and total IgE levels 30-1300 IU/ml [105]. It has been shown effective to reduce the number of exacerbations and to enhance control of allergic severe asthma [106]. It has also been suggested that Omalizumab could be used as an adjuvant to AIT in order to improve the patient's lung functions and asthma control before initiating AIT [103]. Although indication for omalizumab in asthma is reserved for allergic asthma, no available predictive biomarker have been yet identified [107,108].

Allergic comorbidity can also interfere in the choice of a biotherapy for asthma. Dupilumab is an IgG4 human monoclonal antibody binding to the α subunit of the IL-4 receptor (IL-4R α) shared by IL-4 and IL-13 receptor complex, inhibiting both IL-4 and IL-13 mediated signaling pathways [105]. Dupilumab is effective to treat both severe atopic dermatitis and severe asthma [109]. A recent meta-analysis showed that treatment by Dupilumab for atopic dermatitis can interfere with atopic march. During the period of treatment, it reduced the risk of new allergies by 37% vs. placebo. The treatment benefice did not reverse on treatment discontinuation in off-treatment follow up [110].

1.1.2 Nonsteroidal anti-inflammatory drug-exacerbated respiratory disease

Definition:

Nonsteroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD) is characterized by the presence of chronic rhinosinusitis with nasal polyps (CRSwNP), asthma and an intolerance towards aspirin or other cyclooxygenase-1 (COX-1)-inhibiting NSAIDs [111,112]. It was first observed by Widal et al. in 1922, and then by Samter in 1967 who named it “Samter’s Triad” (nasal polyps, asthma and intolerance to aspirin) [113,114]. The prevalence of N-ERD is estimated to be between 0.3% and 0.9% in the general population, 7% in asthmatic patients, 14% in severe asthmatic patients and 10–21% in patients diagnosed with CRSwNP [115–119].

Mechanism:

Arachidonic acid (AA) is a C20 polyunsaturated fatty acid derived from phospholipid hydrolysis at the inner surface of the cell membrane by phospholipase PLA2 [120]. Eicosanoids, which include prostaglandins (PGs), prostacyclins, thromboxanes (TXs), and leukotrienes (LTs), are hormone-like compounds that are involved in a variety of biological processes, including inflammation, platelet aggregation, electrolyte balance, and smooth

muscle contraction [121]. Leukotrienes are synthesized via the 5-lipoxygenase (5-LO)-dependent pathway, whereas PGs, prostacyclins, and TXs are derived from the cyclooxygenase (COX)-dependent pathway. NSAID are members of a therapeutic drug class having analgesic, antipyretic, antiaggregant and anti-inflammatory effect by inhibiting cyclooxygenase. At baseline, inflammation of the respiratory tract is already ongoing in patients with N-ERD/Aspirin exacerbated respiratory disease (AERD). With COX-1 inhibition by any NSAID, the loss of prostaglandin E2 (PGE2) inhibitory control leads to massive release of histamine and generation of cysteinyl leukotrienes by mast cells, an event that is unique to N-ERD. COX-1 inhibition does not block this alternative pathway, which continues unchecked and further generation of cysteinyl leukotrienes (Figure 2). LTs induce the release of interleukin-33 and subsequent mast cell activation, with bronchoconstriction occurring as a result of the direct effects of cysteinyl leukotrienes, prostaglandin D2, and other mast cell-derived products (Figure 3). Prostaglandin D2 recruits effector cells expressing the chemoattractant receptor homologue expressed by type 2 helper T (Th2) cells (CRTH2) to airway tissue and induces bronchoconstriction through T prostanoid receptors.

The pathogenesis of N-ERD is still unclear. The absence of familial clustering argues against a strong genetic basis, and the identification of variants of candidate genes in small studies has not been replicated [122,123]. The combination of genetic susceptibility and external respiratory irritation, such as viral infections and air pollution, remains the main hypothesis for the genesis of AERD [111,124].

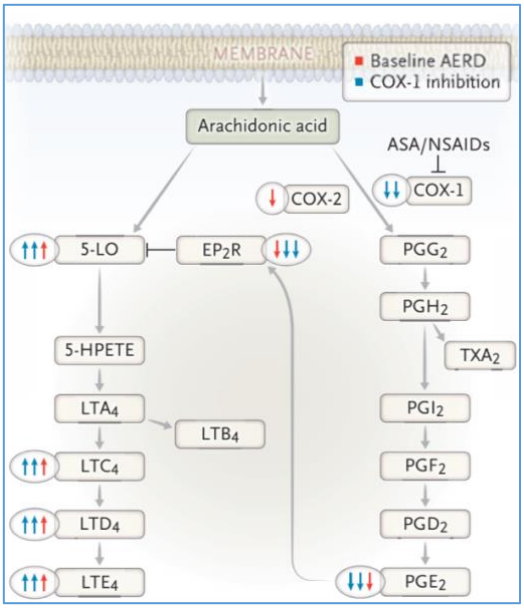

Figure 2 Arachidonic acid metabolism and N-ERD from [111]

Red arrows represent abnormal baseline conditions in patients with AERD, and blue arrows indicate changes after COX-1 inhibition. The number of arrows indicates the magnitude of change. ASA denotes acetylsalicylic acid, EP2R prostaglandin E2 receptor, 5-HPETE 5-hydroperoxyeicosatetraenoic acid, LT leukotriene (types A4, C4, D4, and E4), 5-LO 5-lipoxygenase, PG prostaglandin (types G2, H2, I2, and F2), and TXA2 thromboxane A2.

Clinical presentation:

Patients intolerant to NSAID exhibit reactions involving upper airways (nasal congestion, rhinorrhea, and sneezing) and lower airways (laryngospasm, cough and wheeze). It can less frequently be observed gastrointestinal symptoms (abdominal pain and nausea) and cutaneous symptoms (flushing and urticaria) [111]. The reaction appears 30 to 90 min after an oral intake of NSAID, but intravenous administration provoke reactions earlier within 15 minutes [111,125]. Most patients experience also alcohol related respiratory reaction through unclear mechanism [111,126]. The gold standard for diagnosis of N-RED is the aspirin challenge even though 24 hours urinary leukotriene E_4 measurement has been assessed to be a potential good surrogate [127]. N-ERD is never present at birth, it appears from late childhood to adulthood with a median age of onset of 30 years and a female predominance with a sex ratio

approximately of 2:1 [128–130]. About two third of patients presents atopy [111,130]. Concerning the natural history of the disease, controversial results exist. In a retrospective multicentric study on 500 N-ERD patients, the first symptom of the disease was rhinitis through a flu-like infection, supporting the hypothesis of a viral trigger of the disease [124,129]. However, more recent prospective data on 240 patients reported asthma as the first symptom in 50 % of cases, than polyps or rhinitis in 29 % of cases [131]. Determinants of asthma as first symptom of appearance was early age of onset and higher BMI; rhinitis as first symptoms was associated with later age of NSAID intolerance onset.

N-ERD patients experience a more severe CRSwNP than patients with CRSwNP alone, or CRSwNP and asthma [119]. Indeed, they undergo more sinus surgeries, with more relapse, with a higher probability to develop a corticosteroid dependent disease [119,132]. Concerning asthma outcomes, N-ERD have been associated with poorer respiratory function, more often emergency visit, and more frequent severe exacerbation [133].

Specific Treatment:

Even though polyps recurs rapidly after, polyps surgery treatment have been proven to improve asthma control in addition of controlling sinonasal disease [134]. In twenty-eight N-ERD patients, aspirin hypersensitivity reaction have been observed less severe after nasal surgery with a decrease of FeNO, blood eosinophils and urinary LTE_4 [135].

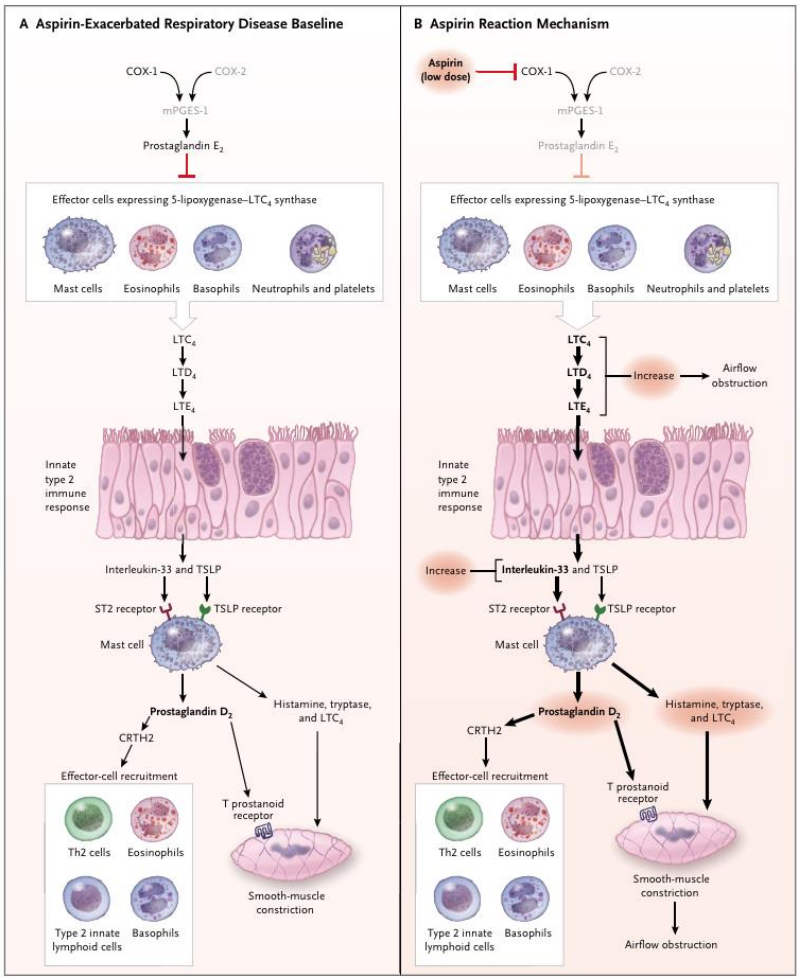

Figure 3 Putative Mechanism of N-RED from [123]

LTD4 designates leukotriene D4, LTE4 designates leukotriene E4, and ST2 designates suppression of tumorigenicity 2.

Aspirin desensitization, or induction of tolerance, is a unique treatment option for N-ERD [136]. Aspirin desensitization is achieved by starting at low dose of aspirin (approximately 40.5 mg), and gradually increasing the dose over a period of 1 to 3 days, during which drug induced reactions become milder and shorter and then disappear. When the target dose of 325 mg is achieved, any additional doses of aspirin or other COX-1–inhibiting NSAIDs do not induce hypersensitivity reactions [111]. Aspirin desensitization has been evaluated efficient to improve asthma control, sinonasal symptoms, quality of life and lung function in double blind controlled

trial [137–140]. Moreover, daily aspirin desensitization has been found effective at slowing the rate of polyp regrowth and reducing the need for repeat sinus surgery [137,141,142]. Nevertheless, aspirin desensitization should be used with caution because of an increased risk of adverse event including major gastrointestinal bleeding, gastritis, asthma exacerbation and severe rash [143].

Concerning anti-asthma monoclonal antibodies, the efficacy on asthma control (Asthma control test, asthma control questionnaire, FEV1) and nasal control have been demonstrated for Omalizumab, Mepolizumab (anti-IL5), reslizumab (anti -IL5), Benralizumab (anti IL-5) and dupilumab [144]. Unfortunately, no head-to-head comparison has been performed. Concerning induction of tolerance, omalizumab has shown to be able to reach aspirin tolerance in more than 50 % of case, and dupilumab to obtain complete and partial tolerance to aspirin in respectively 23.3 and 33.3% of cases [117,145,146].

1.1.4 Exercise induced bronchoconstriction

The term exercise induced bronchoconstriction (EIB) describes transient airway narrowing that occurs during or after vigorous exercise because of large volumes of unconditioned air entering the lower airways to meet increased ventilatory demands [147]. Asthmatic patients can describe symptoms during exercise, EIB patients refer to patients that do not have history of asthma and experience symptoms only during exercise [148,149]. EIB usually develops within 15 minutes after 5 to 8 minutes of intensive aerobic training and usually resolves within 60 minutes [148,150]. The exact mechanisms of EIB have not been established with certainty, but proposed mechanisms include both airway cooling and postexercise rewarming of the airways. Vigorous exercise requires increased ventilation, leading to respiratory water loss and subsequent airway cooling and drying. This leads to an increase of osmolarity in the airways surface liquid and the development of an inflammatory response with the release of mast cell mediators in susceptible

individuals provoking bronchoconstriction [151,152]. Breathing poorly conditioned air at high flows for long periods of time or high volumes of irritant particles or gases could also lead to epithelial injuries and neurogenic inflammation [152].

Prevalence of EIB in children have been estimated to be between 10 to 20 % of the population [153,154]. In athletic adult population, a recent meta-analysis estimated the prevalence of lower airway dysfunction, including EIB and/or asthma, and/or airway hyper-responsiveness. It was estimated to be to 21.8% (95% CI: 18.8-25.0%) with higher prevalence observed in elite endurance athletes 25.1% (CI: 20.0- 30.5%), in those participating in aquatic (39.9%) (CI: 23.4-57.1), and in winter-based sports (29.5%) (CI: 22.5-36.8%) [155]. Athletes in high-ventilation sports are more likely to have EIB symptoms compared with those in low-ventilation sports [148].

The diagnosis of EIB is clinically challenging due to the poor predictive value of self-report respiratory symptoms [156,157]. The first recommended exam is spirometry, which, in case of airway obstruction or airway reversibility, state the diagnosis of asthma [157,158]. In individuals with normal resting lung function and negative bronchodilator responsiveness tests, a form of indirect bronchial provocation (e.g., exercise testing, eucapnic voluntary hyperpnoea, or inhaled mannitol) is recommended to secure a diagnosis [157].

Concerning management of EIB include non-pharmacology therapy such as preexercice warm -up routine that can reduce or prevent EIB in up to 50 % of athlete [148,159,160]. First line of pharmacological therapy is historically the administration of a short-acting beta-2 agonist (SABA) priori to exercise [158]. However regular use of SABA can lead to treatment tolerance and loss of bronchoprotection, that's why the Global Initiative for Asthma (GINA) now opposes the use of SABA in isolation and instead recommends 'as-needed' (i.e., symptom-driven) or a daily combined low dose of ICS + fast long-acting beta-2-agonist (LABA) as first-line therapy

[13]. Other pharmacological therapies for EIB include leukotriene receptor antagonists, anticholinergics and mast cell stabilizing agents [157].

We have seen that phenotypes based on the type of trigger can distinguish groups of individuals with distinct clinical forms, specific mechanisms and treatment response. Occupational asthma can be elicited by multiple agents. It can be hypothesized that the diversity of the agents may contribute to different phenotypes.

1.2 Clinical phenotypes

Asthma has multiple clinical presentations. The age of onset, the diseases associated, the severity may differ. Some clinical presentations underline causative effect of environmental exposures in predisposed subjects and also determine specific pathophysiologic pathways, such as obesity related asthma. Some additional clinical manifestations, accompanied by associated comorbidities, underscore the necessity for a more comprehensive approach to the treatment of asthmatic patients who will not achieve complete resolution of their respiratory symptoms through the use of conventional anti-asthmatic therapies alone. In both cases, it is important to address a good characterization of the disease to provide the best treatment.

1.2.1 Obesity related asthma

The prevalence of obesity in France has been estimated at 15.8% and 15.6% in men and women respectively [161]. From 1990 to 2022, the percentage of children and adolescents aged 5–19 years living with obesity increased four-fold from 2% to 8% globally, while the percentage of adults 18 years of age and older living with obesity more than doubled from 7% to 16% [162]. Obesity has been proven to be a risk factor of asthma. A meta-analysis was performed on seven prospectives studies gathering 333,102 adult participants for which overweight and obesity

were described associated with the incidence of asthma (OR of 1.51 95% CI, 1.27–1.80). A dose– response effect of elevated BMI on asthma incidence was observed; no differences on gender was noticed [163]. In children and adolescent too, obesity is recognized as an independent risk factor for asthma on the basis of extensive epidemiological evidence form several prospective cohorts [164,165].

Multiple mechanisms have been proposed to explain the relationship between asthma and obesity. Lung function is impacted by obesity. Excessive accumulation of fat in the thoracic and the abdominal cavities leads to lung compression and a reduction of lung volumes [166]. Also, in obese asthmatic subject, the collapsibility of distal peripheral airways has been showed to be determined by obesity and to reduce with weight loss [167]. Obesity has been described as associated with airway hyperresponsiveness in large prospective cohorts even though the association was not replicated in smaller studies [168–170].

A genetic predisposition, and epigenetic factors have been also suggested as involved in obese related asthma phenotype. Candidate gene studies have identified a few genes associated with asthma and BMI, such as protein kinase C alpha, leptin, beta-3 adrenergic receptor, and DENN domain containing 1B [35]. For example, genetic variant on chromosome 17q21 regulates orosomucoid-like 3 (ORMDL3) expression and contributes to the risk of childhood onset asthma [171,172]. Also, a gene-by-environment analysis found 7 Single-nucleotide polymorphisms (SNP) in the latter locus 17q21, which were associated with BMI only among subjects with asthma, in 2 independent cohorts [173]. More recently, a substantial positive genetic correlation between BMI and later onset asthma have been found in a cross-trait genome-wide association study [174].

Diet has been underlined as being a possible factor in the association between obesity and asthma. The impact of diet on respiratory health is a recent research topic [175]. Western diet has been associated with the development of asthma, whereas mediterranean diet would have a

beneficial effect [176]. Vitamine D deficiency has been observed in different work as associated respectively with obesity and with asthma exacerbation but interventional studies remain inconclusive [177–179]. In children, high consumption of beverage containing high sugar level have been observed as associated to an increased probability of being asthmatic [180]. Diet associated with obesity, as high in saturated fatty acids ones, have been shown to increase neutrophilic inflammation and to suppress bronchodilatator recovery in non-obese and obese asthmatic subjects [181]. Low fiber diet is associate with gut microbiome poor in short chain-fatty acid [170]. The level of short-chain fatty has been shown to modulate allergic airway disease with a protective effect of high fiber diet [182].

Metabolic and immune changes are factors contributing to the syndrome of obesity related asthma. Metabolic syndrome and insulin resistance have been demonstrated as associated with asthma [183–185]. Adipokines and other cytokines produced or induced by adipose tissue can affect asthma outcomes [170,186]. Adipose tissue secretes leptin that has been measured higher in visceral adipose tissue in obese asthmatic subject than in non-asthmatic obese subjects [187]. Visceral fat leptin level has been reported to correlate with airway hyperresponsiveness and leptin concentration in plasma was proven to correlate inversely with FEV1, FVC and FEV1/FVC ratio in obese adolescent [187–189].

Obese related asthma is characterized by being a more severe phenotype. In The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study, which is a large prospective observational cohort of patients with high asthma medication in the United States, nearly 60 % of adults are obese [190]. Obese asthmatic patients have worse asthma control, lower quality of life and do not respond as well as non-obese asthmatics to standard controller medication [191,192]. Observations have been made of differences in the clinical characteristics of obese patients in relation to the age of onset of asthma [193]. Compared to late-onset obese asthmatics, early onset obese asthmatics exhibits more airway

hyperresponsiveness, more severe exacerbations, and eosinophilic inflammation [193]. Late onset obese asthma are described as being more often female predominant, non-atopic, with neutrophilic inflammation [193–195]. It has been stated that obesity can both induce asthma and also be a comorbidity that complicate asthma management, asthma having be suggested as a risk factor for obesity [170,196].

Multiple interventions have been evaluated in order to provide specific management concerning obese-related asthma. Weight loss in obese asthmatic patients has been proven to enhance asthma control, improve lung function and quality of life [197–199]. Also, weight loss inferior to 10% of total weight has been shown to be inefficient to induce clinically meaningful improvement on asthma [198]. Association of exercise training and dietary intervention has been proved more efficient in term of clinical control, aerobic capacity and in term of weight loss [197]. Bariatric surgery has been proven to be efficient to improve asthma control, to reduce asthma medication intake and to reduce exacerbation rate in obese asthmatic patients [170,200,201].

To sum up, obese related asthma is an increasing source of concern for physician because of the evolution of worldwide incidence of obesity. Multiple mechanisms have been proposed to explain the association between obesity and asthma as well as specific management by weight loss intervention. Nevertheless, even in obese related asthma, different phenotypes seem to coexist notably in function of the age of onset. Obesity has been described as a comorbidity that can increase severity of asthma and also a distinct cause of asthma [195].

1.2.2 Inducible laryngeal obstruction: an example of asthma comorbidity

Inducible laryngeal obstruction (ILO) describes an inappropriate, transient, reversible narrowing of the larynx in response to external triggers [202]. Typical clinical features include

wheeze, dyspnea and cough, and these symptoms are highly variable and evanescent. The laryngoscopy with provocation is the gold standard for diagnosis [203]. ILO can mimic asthma but it can be also an associated comorbidity; the prevalence of ILO has been estimated to be up to 25 % in asthmatic population [204]. ILO belongs to a larger entity, laryngeal dysfunction syndrome, that gather functional laryngeal diseases, refractory cough and muscle tension dysphonia, that are over-represented in asthmatic patients [205–208]. ILO is associated to multiple factors as laryngeal mechanical insufficiency, psychological contribution and neuronal dysfunction [202]. Airway neuronal dysfunction, measured by capsaicin challenge, has been estimated to be more prevalent in asthmatic patients than in non-asthmatic subjects, especially in female non atopic asthmatic subjects [209]. Laryngeal dysfunction syndrome could be predominant in a particular subset of asthmatic patients. In difficult to treat asthmatic patients, the coexistence of induced laryngeal obstruction have been described to be associated to distinct characteristics as preserved lung function, dysfunctional breathing and lower blood eosinophilic count [210]. Diagnosis of ILO is important to avoid unnecessary anti asthmatic treatment and also to provide specific management. Speech therapy, which is the first line therapy for ILO, has been suggested to improve control of asthma and to reduce medication intake in patients exhibiting asthma and ILO [211–213]. Likewise, refractory chronic cough, which causes a high burden in term of quality of life and in term of over-treatment in asthmatic population, is a clinical trait whose management is going to be enhance by new specific therapies as gefapixant [214,215]. Other treatable comorbidities have been associated with poorer asthma outcomes including dysfunctional breathing and chronic rhinosinusitis [216].

To summarize, categorizing individuals with asthma in function of clinical presentation may be important for two reasons:

- First it can identify factors that can be causal of the disease like obesity.

- Secondly it can outline comorbidities, which may affect the clinical intensity, the severity and treatment response.

In each case, the careful characterization of the clinical presentation may provide precise therapeutics and is to be performed. We could hypothesize that its characterization may also unveil therapeutic possibilities.

1.3 Inflammatory phenotypes

Airway inflammation is a critical element in the definition of asthma [13]. Soon, eosinophilic inflammation have been associated to response to both oral and inhaled corticosteroids [33,217]. Research on airway inflammation has provided molecular framework for elucidating underlying mechanisms of asthma, new therapies selecting precise pathways and biomarkers allowing to predict treatment response.

1.3.1 Inflammation and asthma

The different type of airway inflammation in asthma are illustrated in figure 4. Two main types of airway inflammation have been described, T2 high and T2 low inflammation. T2 high asthma gathers allergic asthma, that has already been described in figure 1 and eosinophilic asthma. In eosinophic asthma, secondary to the exposure to pollutants, viruses, or bacteria, airway epithelial cells release alarmins such as interleukin-25, interleukin-33, and thymic stromal lymphopoietin (TSLP). Alarmins released by epithelial cells and eicosanoids (i.e., cysteinyl

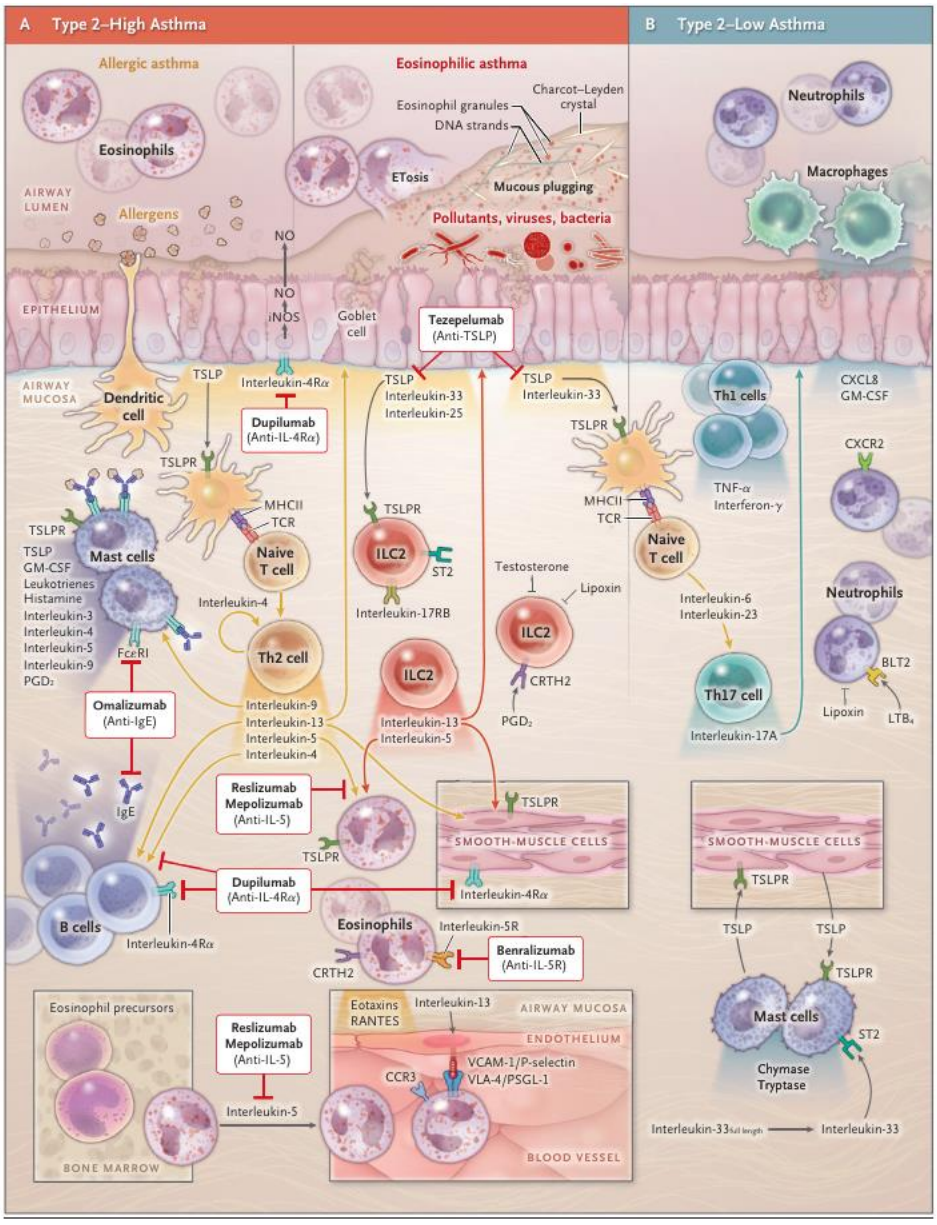

Figure 4 Airway inflammation in asthma and targets of biologic therapies. From [241]

leukotrienes C4 and D4 and prostaglandin D2) activate type 2 innate lymphoid cells (ILC2), which lack antigenspecific TCRs but express receptors for these alarmins and leukotrienes. ILC2 produce high amounts of the type 2 cytokines interleukin-5, and interleukin-13. Interleukin-5 promotes the proliferation and differentiation of eosinophils from bone marrow eosinophil progenitors, prolongs eosinophil survival, and activates eosinophils, which release cysteinyl leukotrienes and toxic granules, causing tissue damage, aggravating chronic airway inflammation, and leading to acute exacerbations of asthma. Interleukin-4 and interleukin-13 induces expression of the enzyme inducible nitric oxide synthase (iNOS) in epithelial cells, leading to an increase in fractional exhaled nitric oxide (FeNO); interleukin-13 also elicits mucous hypersecretion and stimulates contraction of airway smooth-muscle cells, causing bronchoconstriction. Interleukin-4 and interleukin-13 play an important role in recruiting eosinophils from the blood circulation to the airway mucosa both directly, by enhancing the expression of adhesion molecules on endothelial cells, and indirectly, by eliciting the production of chemokines such as eotaxins by epithelial cells. Through eosinophil extracellular traps, Charcot–Leyden crystals, and eosinophil peroxidase–generated oxidants, airway eosinophils mediate mucous plug formation and contribute to chronic airflow obstruction in type 2–high severe asthma.

Type 2–low asthma encompasses paucigranulocytic asthma and neutrophilic asthma. Type 1 helper T (Th1) and type 17 helper T (Th17) CD4+ lymphocytes may stimulate neutrophilic inflammation through tumor necrosis factor α (TNF-α), interferon-γ, interleukin-6, interleukin-17A, and CXCL8 (CXC motif chemokine ligand 8). The alarmins TSLP and interleukin-33, the latter activated from full-length interleukin-33 by mast-cell–derived tryptase, may be involved in the crosstalk between mast cells and airway smooth-muscle cells, contributing to airway hyperresponsiveness. Paucigranulocytic asthma is defined by asthma without evidence of elevation of inflammatory cells in airways.

A number of techniques exist for the assessment of airway inflammation. Bronchoalveolar lavage and endobronchial biopsy represent the gold standard for identifying airway inflammation phenotypes. However, these tests are invasive and expensive. Inflammatory cells count in induced sputum has been proven to be a safe, reproducible and accurate surrogate to these techniques [218–220]. Sputum is induced by inhalation of nebulized hypertonic solution and then the sample is processed in order to realize cellular count [221]. Eosinophilic and neutrophilic airway inflammation corresponds an isolated excess of these cells. In contrast, mixed granulocytic inflammation denotes an excess of both eosinophilic and neutrophilic cells. Paucigranulocytic inflammatory phenotype is used to describe the absence of an excess of both eosinophils and neutrophils. However induced sputum technique is only available in specialist centers, it is time consuming and in 20 % of cases, patients are not able to produce suitable samples [222]. Other non-invasive methods have been proposed as FeNO measurement, serum periostin and blood eosinophil count and total IgE. Even though these biomarkers are more easy to perform, they only estimate eosinophilic inflammation, without a high accuracy comparatively with induced sputum [223]. In this thesis work, we will stress the point of inflammatory phenotype based on induced sputum.

Via induced sputum technique, inflammatory patterns have been associated with clinical phenotypes. Table 1 display a summary of these studies. The threshold to define each inflammatory pattern vary between study (for eosinophilic inflammation between 1.9 and 3%, for neutrophilic inflammation between 40 and 76%), which affects the prevalence of each inflammatory pattern. Eosinophilic inflammation is present in around 50 % of asthmatic patients and neutrophilic inflammation in around 20% [224,225]. Paucigranulocytic pattern is associated with highest lung function, better control, less severity [225,226]. Eosinophilic inflammation has been associated to poor lung function, high non-specific hyperresponsiveness (NSBH), and uncontrolled disease [225–228]. Neutrophilic inflammation has been linked with

older age, obesity, female gender, poor response to ICS, poor lung function, and severe disease [226,228–232]. Longitudinal studies have respectively suggested that mixed granulocytic pattern and high variation of eosinophils count could be associated to low function decrease [233,234]. A high variation of eosinophils have also been proven to be linked to a higher risk of healthcare use [234].

Table 1 Inflammation pattern ascertained by induced sputum and clinical characteristics in asthmatic patients

Reference	Type of study	Cut- off for inflammatory profile	Population	Prevalence	Main results
Turner MO et al. [227]	Cross-sectional	Eosinophilic ≥ 4%	Subject with asthma during mild exacerbation N=50	Eosinophilic 53% Non eosinophilic 47%	FEV1 lower in Eosinophilic group (mean: 70 vs. 88%) Negative correlation sputum eosinophils and FEV1 (r= -050, p=0 002) and PC20 (r= -040).
Janatakon A et al. [235]	Cross-sectional	NA	Mild asthma (no ICS), n=23 Moderate asthma (ICS), n=16 Severe asthma (OCS), n=16 Control, n= 12	NA	Increased sputum neutrophil count in severe asthma vs. mild asthma (median: 53 vs. 35 %)
Louis R et al. [229]	Cross-sectional	NA	Intermittent asthma, n= 19 Mild to moderate asthma, n=38 Severe persistent asthma ,n=17 Control subjects ,n=22	NA	Absolute eosinophilic count inversely correlated with PC 20 (r=-0.55) and FEV1 (r=-0.43) Severe asthmatic on OCS had fewer neutrophils (absolute count) than Severe asthmatic without OCS Absolute neutrophilic count inversely correlated with PC 20 (r=-0.34) and FEV1
Woodruff P et al.[228]	Cross-sectional	NA	205 asthmatic subjects Mean age: 33 y	NA	Adjusted association between eosinophils and neutrophils percentage with FEV1 (ß= -0.38 and -0.11) Adjusted association between eosinophils percentage with PC 20 (FEV1 ß= -0.01) Percentage of eosinophils lower in subjects treated by ICS (median 1.6 vs. 3.1%) than in untreated participants
Green R et al [230]	Cross-sectional Subgroup with evaluation before/after treatment	Isolated neutrophilic : Eosinophils : <1.9 % Neutrophils : >65.3%	Intermittent asthma, n=143 Persistant asthma, n=116 Control subjects, n= 34 49 subjects studied before and after 2 months of treatment by inhaled budesonide	Isolated neutrophilics: 23%	Isolated neutrophilics asthmatics were older, more likely female, non-atopic than other asthmatics Longitudinal subgroup analysis: less improvement in asthma control, FEV1 and PC20 in isolated neutrophilic asthmatics (n=11)
ENFUMOSA study [231]	Cross-sectional	NA	Mild asthma (controlled with ICS), n=158 Severe asthma, n=163	NA	Severe asthma patients were older, more frequently female, with higher BMI, lower FEV1 and higher neutrophilic count vs. mild asthma: 61.2% vs. 57.3%
Simpson et al. [224]	Cross sectional Subgroup with longitudinal analysis	Eosinophilic: >1.01% Neutrophilic: >61% Mixed graulocytic: both Paucigranulocytic: none	93 asthmatic subjects 40 asthmatic subjects with a second visit after 5 years	Eosinophilic: 41% Neutrophilic: 20% Paucigranulocytic: 31% Mixed granulocytic: 8%	Neutrophilic and mixed granulocytic older than paucigranulocytic and eosinophilic asthmatic patients Long term reproducibility of the classification in longitudinal analysis: kappa 0.64 (moderate agreement)
Hastie et al. [226]	Cross-sectional	Eosinophilic: ≥2% Neutrophilic: ≥40% Mixed graulocytic: both Paucigranulocytic: none	242 asthmatics subjects with 48 severe asthmatics	Eosinophilic: 24% Neutrophilic: 29% Paucigranulocytic: 36% Mixed granulocytic: 11%	Mixed granulocytic asthmatics: lowest lung function Neutrophilic and mixed granulocytic asthmatics: oldest patients, more severe exacerbation Eosinophilic and mixed granulocytic asthmatics: more uncontrolled patients

Reference	Type of study	Cut- off for inflammatory profile	Population	Prevalence	Main results
Schleich et al. [225]	Cross-sectional	Eosinophilic: ≥3% Neutrophilic: ≥76% Mixed graulocytic: both Paucigranulocytic: none	508 unselected asthmatics subjects	Eosinophilic: 46% Neutrophilic:18% Paucigranulocytic: 40% Mixed granulocytic: 3%	Paucigranulocytic asthmatics: higher FEV1 and FEV1/FVC than in other groups Eosinophilic asthmatics: more frequent atopy, lower asthma control, higher total IgE, FeNO, higher NSBH vs. paucigranulocytic asthmatics Mixed granulocytics: lowest function, highest NSBH Adjusted association between FEV1/FVC with eosinophilic sputum count (ß=-0.039)
Newby et al. [233]	Longitudinal study Follow up during 6 years	NA	97 severe asthmatic patients Cluster analysis in function of lung function decline	NA	Decrease of lung function was associated with eosinophilic variability in time: Eosinophilic with high variation was at greater loss of FEV1 than hypereosinophilic with no variation and non-eosinophilic with no variation.
Moore et al. [232]	Cross sectional Cluster analysis	Eosinophilic: ≥2% Neutrophilic: ≥40% Mixed graulocytic: both Paucigranulocytic: none	423 asthmatic patients from the Severe Asthma Research Program	NA	Percentage of neutrophils was assessed to be an important variable in cluster analysis by stepwise discriminant analysis Neutrophilic inflammation was more prevalent in cluster C and D: older patients, oldest age of onset, high dose of ICS, high rate of hospitalization.
Wang et al. [236]	Cross -sectional	Eosinophilic: ≥3% Neutrophilic: ≥40% Mixed graulocytic: both Paucigranulocytic: none	256 outpatients' clinics from the severe Asthma Web based Database 146 controlled, 90 uncontrolled asthma	Eosinophilic: 26.6% Mixed Granulocytic: 1.3%, Neutrophilic: 11.4% Paucigranulocytic: 60.6%	Uncontrolled asthmatic patients exhibited higher sputum eosinophilic count [median IQR 1.68% (0.0, 17.1%) vs. 0.2% (0.0, 1.3%)]
Ntonsi et al. [237]	Cross sectional	Eosinophilic: ≥3% Neutrophilic: ≥60% Mixed graulocytic: both Paucigranulocytic: none	240 asthmatic patients for two tertiary hospitals	Eosinophilic: 40% Mixed Granulocytic: 6.7%, Neutrophilic: 5.4% Paucigranulocytic: 47.9%	Paucigranulocytic asthma: better lung function than other groups Severe refractory asthma more frequent in eosinophilic and mixed granulocytic patients than neutrophilic and paucigranulocytic. 14.8% of patients with pauci-granulocytic asthma had poor asthma control.
Hastie et al. [234]	Longitudinal analysis over 3 years with visit each year	Eosinophils predominantly<2%, ≥2%, or highly variable eosinophil neutrophil predominantly<50%, ≥50%, or highly variable	206 asthmatic patients in seven clinical sites	Predominantly high eosinophils: 25% Predominantly low eosinophils: 59% Highly variable eosinophils: 16%	Predominantly low eosinophils: better FEV1 and FEV1/FVC vs. Highly variable and predominantly high eosinophils Highly variable eosinophils group: greater healthcare use Predominantly high-neutrophil group: greater age and year since diagnosis than highly variable and predominantly low neutrophil Subjects with both predominantly high eosinophils and neutrophils: greater loss of lung function

Inflammatory pattern is predictive of treatment response. An increase of sputum eosinophil has been observed to be predictive of asthma exacerbation [238]. Management strategy of asthma directed at normalization of the induced sputum eosinophil count or FeNO has been proven to reduce asthma exacerbations [239,240]. Multiple biologic agents have been developed targeting T2 inflammation pathway [241]. For each biotherapy, T2 biomarkers, especially blood eosinophils count, have been found to be predictive of a clinical response [241–243].

Even though inflammatory patterns became more and more important in the understanding and the classification of asthma, information on the relationship between inflammatory patterns and occupational asthma outcome remain sparse and discordant [244–251].

1.4 From endotype to treatable traits

We have shown that each asthma characterization according to trigger, clinical form and inflammatory pattern has enabled us to highlight specific asthma management approaches. Nevertheless concerns were risen about lack of specific cellular biomarkers for asthma phenotypes and the subjectivity of these phenotypes [28,252]. A robust system of classification that incorporates the multidimensionality of asthma has been suggested to be able to provide a framework for identifying distinct phenotypes with specific pathophysiologic abnormalities that predict response to particular therapy [253,254]. These latter potential phenotypes, were defined as endotypes, contraction of endophenotype, by Anderson in 2008 [255]. An endotype is a disease subtype defined functionally and pathologically by a molecular mechanism or by response to treatment. Endotype concept have been elaborated in order to overpass the T2-inflammation hypothesis, which was judged inadequate to understand the substantial heterogeneity of asthma [255]. In order to deal with asthma heterogeneity, cluster analysis were performed, intending to propose unbiased classifications, combining clinical and molecular dimensions.

Kaur et al. have summarized main cluster studies outcome [256] (Figure 5). Based on the age of onset, lung function and allergic status, four main phenotypes, have been identified: (1) early-onset allergic asthma, (2) early-onset allergic moderate-to-severe remodeled asthma, (3) late-onset nonallergic eosinophilic asthma, and (4) late-onset nonallergic noneosinophilic asthma. Other features, commonly linked to asthma as sex, obesity and smoking, were less consistent across these studies [253,257–259]. Only few studies have evaluated the longitudinal stability of phenotypic clusters. Boudier et al. demonstrated a strong stability of clusters over 10 years using latent transition analysis [260]. Cluster analysis was applied several years apart in the same population and the structure of the phenotypes obtained was shown similar. After 20 years of follow up, cluster determination at baseline were associated with differential asthma outcome

[261]. Up to now, phenotypes identified from unsupervised studies cannot be called endotypes as they are not defined functionally and pathologically by a molecular mechanism or by treatment response.

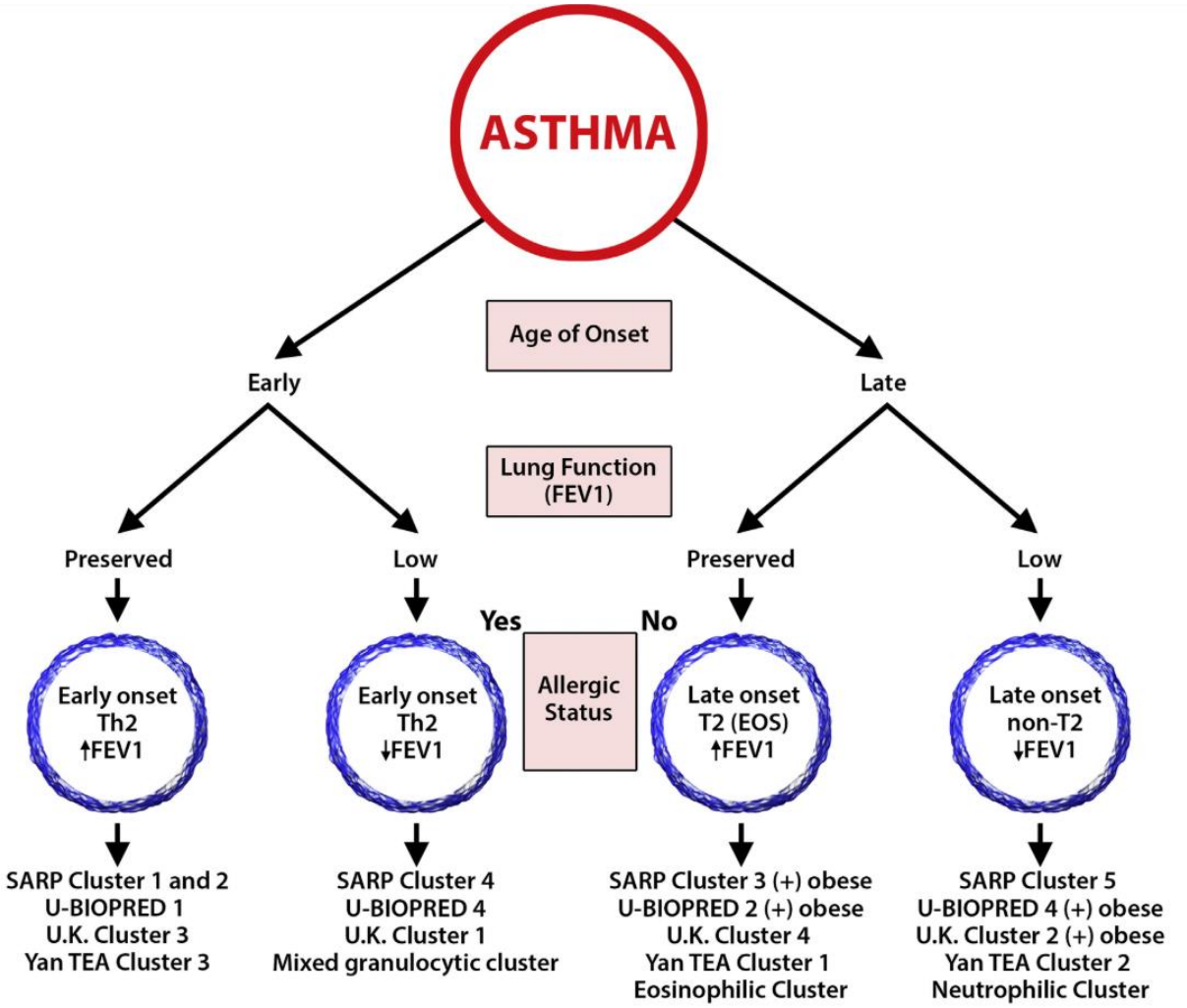

Figure 5 Similarities in cluster studies, from [256]

Lötvall *et al*. proposed rules for defining asthma endotypes [23]. They defined 7 parameters to define each potential endotype: clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology and treatment. By group consensus they proposed that in order to be labelled endotype, a phenotype should fulfill at least 5 of the 7 parameters and should be validated in prospective studies. Some parameters were considered unsuitable for defining endotypes: 1) Disease severity, which can be caused by several factors as intrinsic disease

activity, concomitant comorbidity or patient adherence to treatment, 2) comorbidities, because they exert influence on phenotypes rather than the endotype. From this definition 6 endotypes, whose robustness needed to be confirmed by prospective clinical studies, were proposed: N-ERD, allergic bronchopulmonary mycosis, allergic asthma, severe late-onset hypereosinophilic, allergic preschool wheezers, and EIB. As authors declare, endotype might be a good framework to facilitate future research to establish genetic association, identify biomarkers for disease endotypes, and test novel therapeutic target and endotype specific treatment. The endotype framework is based on a strong holistic hypothesis: each asthma variations can be resumed to singular inflammatory molecular pathways that are to be discovered in order to be cured.

Classification shapes medicine and guides its practice [7]. In 2018, the Lancet Commission convened experts in various fields, unified by a shared expertise in asthma, to deliberate on the optimal conceptualization and management of asthma in the 21st century [262]. They stated that over the past 50 years, two main eras of asthma management have been identified. The first of these was the bronchodilatator era, which began in the mid-1960s and focused on airway hyperresponsiveness. The second was the anti-inflammatory era, which began in the late 1980s and involved the more aggressive use of inhaled corticosteroids. They suggested that the latter era perpetuated the myth that airway inflammation was the origin of all asthma troubles. They rose the important question of whether the phenotypic heterogeneity of asthma can be explained by discrete mechanistic endotype. Indeed, phenotypic traits (ie, symptoms and airflow obstruction) can be caused by multiple disease mechanisms [262,263]. The Lancet commission supports a more reductionist approach of classification, reductionism being a epistemological concept stating that the whole is the sum of its part [264]. In 2016, Agusti et al. proposed a new paradigm shift with the introduction of the treatable traits framework. [12,262].

A treatable trait is a phenotypic or biologic characteristic, that can be assessed and successfully targeted by therapy to improve a clinical outcome in a patient with airway disease. Treatable traits must share three characteristics: 1) *clinical relevance*, which means they are linked to a relevant clinical outcome such as exacerbations; 2) *detectable* through validated "trait identification markers" (eg. Presence of emphysema on computed tomography); 3) *treatable*, meaning that effective treatment is available and accessible [265,266]. Treatable traits can co-exist in the same patient. This strategy recognizes the clinical and biological complexity of airway disease and acknowledges that both clinical phenotypes and endotypes can occur in isolation or in combination in any patient and might change over time, either as part of the natural history of the disease or because of therapy. Also, authors insist that no causal relationship should be assumed between one component and another [12,262]. There are many potential treatable traits in chronic airway diseases which can be ordered into three domains, namely pulmonary traits, extrapulmonary traits and risk factor/behavioral traits [12,265,266]. Treatable traits proposition has varied over time, the latest proposition is illustrated table 2.

The treatable trait strategy encompasses overlapping disorders, such as COPD/asthma overlap. The definition of diseases may be unduly restrictive, implying a mechanistic pathway that leads to a step-therapy approach that benefits mostly to stereotypic patients and results in over-treatment in many. The Lancet Commissioners considered that asthma should solely become as a descriptive label for a collection of symptoms [262].

The treatable trait strategy rallies comorbidities, environmental and lifestyle factors, and places emphasis on the consideration of these in patients with persisting morbidity despite effective intervention against pulmonary treatable traits [262,267]. Even though further studies are needed, targeting treatable trait in severe asthma has been proven efficient in term of quality of life and asthma control compared to usual care in a randomized controlled trail [268].

Interestingly, in the treatable trait framework, occupational exposures are considered as a behavioral/risk factors traits of respiratory diseases. We have seen that the definition of occupational asthma resides in the causality of occupational exposures on asthma. Does occupational asthma exhibit specific endotypes of asthma, or occupational exposures are behavioral treatable traits with no relation with pathophysiological mechanisms?

In less than a century, since the first modern classification by Rackemann, our understanding of the heterogeneity of asthma disease has greatly evolved. Characterization of asthma in term of trigger and clinical presentation has enable to identify specific mechanisms and also comorbidities that can aggravate the disease and affect treatment response. The advent of inflammatory pattern characterization has led to a holistic approach, the endotype framework, which states that the heterogeneity of the disease can be explained by distinct inflammatory pattern that remains to be discovered in order to increase efficiency of treatment. In reaction, the treatable trait framework, more reductionist, states that each composant of the heterogeneity of the disease, gathered in three categories, pulmonary, extra pulmonary and behavioral may be treatable and should be considered independently of causality assumption. The second part of this PhD work will expose the classification of occupational asthma, how the characterization of the disease was made, in which criterion.

Table 2 List of potential treatable traits adapted from [265]

Pulmonary traits	Trait identification marker/diagnostic criteria	Possible treatments
Airflow limitation	FEV1/FVC<0.7 and FEVA<80%	Bronchodilatators
Systemic allergic inflammation	Elevated serum specific IgE	Anti-Immunoglobulin E monoclonal antibody therapy, allergic avoidance, immunotherapy
Dyspnoea	Dyspnoea score ≥ 2, modified Merdical Research Council scale	Pulmonary rehabilitation, breathing retraining
Emphysema	Chest computed tomography, plethysmography, lung compliance	Smoking cessation, lung volume reduction surgery, lung transplantation, α- anti trypsin replacement if deficient
Airway inflammation (eosinophilic)	Sputum eosinophils≥3% and/or FeNO ≥25 ppb and/or blood eosinophils≥0.3 x 10^9/L	Corticosteroids, anti-interleukin-5,-13,-4 monoclonal antibody therapy
Pulmonary hypertension	Doppler echocardiography, brain natriuretic peptide, right heart catheterization	Oxygen therapy, pulmonary vasodilatator therapy, lung transplant
Bronchiectasis	High resolution chest computed tomography	Physiotherapy, mucociliary clearance techniques, macrolides, pulmonary rehabilitation, vaccination
Bacterial colonization	Presence of a recognized bacterial pathogen in sputum (sputum culture, quantitative PCR)	Antibiotics and tailored antibiotic written action plan for infections
Airway inflammation (neutrophilic)	Sputum neutrophils ≥ 61%	Macrolides, tetracyclines, roflumilast
Cough reflex hypersensitivity	Capsaicin challenge, cough count, cough questionnaire	Speech pathology, intervention, gabapentin
Mucus hypersecretion	Volume ≥25 ml of mucus produced daily for the past week in the absence of an infection	Mucociliary clearance techniques with a physiotherapist, inhaled hypertonic salin, macrolides
Hypoxemia	PaO2 ≤ 55 mmHg; PaO2 56-59 mmHg and evidence of complication of hypoxaemia	Domiciliary oxygen therapy

Extra Pulmonary Traits	Trait identification marker/diagnostic criteria	Possible Treatments
Depression	Questionnaires (e.g., HADS depression domain score ≥8, GADS score >5), psychologist/liaison psychiatrist assessment	Cognitive behavioural therapy, pharmacotherapy
Anxiety	Questionnaires (e.g., HADS anxiety domain score ≥8), psychologist/liaison psychiatrist assessment	Pharmacotherapy (i.e., anxiolytics/antidepressants), breathing retraining, CBT
Dysfunctional breathing	Nijmegen Questionnaire Total score ⩾23, B-PAT (breathing pattern assessment tool) score >4	Breathing retraining
Decreased bone mineral density (osteoporosis)	T-score ⩽−2.5	Pharmacotherapy based on osteoporosis guidelines, Vitamin D supplementation, resistance training
Overweight/obesity	Overweight: BMI 25–29.9 kg·m−2, Obesity: BMI ≥30 kg·m−2	Caloric restriction, exercise, bariatric surgery, pharmacotherapy
Sarcopenia	Appendicular skeletal muscle mass index. Males: <7.26 kg·m−2, females: <5.45 kg·m−2	Diet (high protein), resistance training
Deconditioning	Cardio-pulmonary exercise testing, 6MWT	Structured exercise program, rehabilitation
Rhinosinusitis	History and examination, imaging (sinus computed tomography), Sino-Nasal Outcome Test (SNOT- 22)	Topical corticosteroids, leukotriene receptor antagonists, antihistamines, biologics for chronic rhinosinusitis with polyps, surgery, intranasal saline lavage
Induced laryngeal obstruction	Questionnaires (i.e., e.g., Pittsburgh ≥4), laryngoscopy, dynamic neck CT, inspiratory flow– volume curve	Speech pathology intervention, laryngeal botulinum toxin, gabapentin/pregabalin, psychology/psychiatry
Anaemia	Males: Hb < 140 g·L−1, females: Hb < 120 g·L−1	Haematinic (iron/B12) supplementation
Cardiovascular disease	Doppler echocardiography, Electrocardiogram, brain natriuretic peptide	Pharmacotherapy (β-blockers, diuretics, angiotensin-converting enzyme inhibitors), surgery
Gastro-oesophageal reflux disease	Questionnaires, gastrointestinal Endoscopy, pH monitoring	Anti-reflux lifestyle measures, antacids, proton pump inhibitors, fundoplication surgery
Obstructive sleep apnea	Questionnaires (i.e., STOP-Bang Questionnaire), polysomnography	Continuous positive airway pressure, mandibular advancement splint, positional therapy, weight loss

Behavioral/Risk Factors Traits	Trait identification marker/diagnostic criteria	Possible Treatments
Sub optimal inhaler technique	Direct observation and standardized assessment checklists, assessment via chipped inhalers.	Education including demonstration and regular reassessment
Sub optimal adherence	Prescription refill rates, self-reported use of <80% of prescribed medication, chipped inhalers, FeNO suppression test, measurement of drug concentrations	Self-management support, education, simplification of medication regime (i.e., reduce number of medications, frequency of doses and number of devices)
Smoking	Self-reported current smoking, elevated exhaled carbon monoxide, urinary cotinine	Smoking cessation counselling +/- pharmacotherapy
Side-effects of treatments	Patient report, Monitored withdrawal	Optimisation of treatment, alternative therapy, change device
Absence of a written action plan	Patient does not possess a written action plan, or reports not using the prescribed plan during exacerbations	Individualised self-management education with a written action plan
Exercise intolerance	<350 m on 6MWT	Pulmonary rehabilitation
Physical inactivity and sedentary behaviour	Actigraphy, International Physical Activity Questionnaire	Pulmonary rehabilitation, physical activity, breaking bouts of sedentary activity
Sarcopenia	Appendicular skeletal muscle mass index. Males: <7.26 kg·m−2, females: <5.45 kg·m−2	Diet (high protein), resistance training
Exposures (Occupational/ Indoor coal/biomass)	History, Radio allergen absorbance test, skin-prick testing, Exhaled concentration of carbon monoxide	Avoidance where possible
Frequent β2 use	History	Self-management education

Second part:
Phenotype of occupational asthma

The second part of the introduction will focus on occupational asthma, defining it and describing the differences between sensitizer-induced asthma and irritant-induced asthma. We will focus on the different phenotypes that have been described and their pathophysiology, before outlining the current limitations of the state of the art.

2.1 Definition of occupational and work-related asthma

Occupational exposure can cause or exacerbate asthma [269]. Work-related asthma encompasses work-exacerbated asthma (WEA), which is preexisting asthma triggered by work, and occupational asthma (OA), which is asthma caused by work [270] (Figure 6). Work-related asthma has been estimated to represent 25 % of asthma cases [271]. Work-related asthma is associated with impaired quality of life and worst work productivity than non-work-related asthma [272]. Patients with work-related asthma may also experience loss of income and present higher unemployment rate because of their disease [272,273].

The term work exacerbated asthma (WEA) is used to describe the worsening of preexisting or coincident (adult new-onset) asthma because of workplace environmental exposure [274]. The definition of WEA has been developed in 2011, through an Official American Thoracic Society Statement [275]. For any individual, OA and WEA are not mutually exclusive, meaning that someone with OA can subsequently experience WEA, and vice versa [275]. Four criteria have been proposed to define WEA:

1. Pre-existing or concurrent asthma. “Pre-existing asthma” is asthma with onset before entering the worksite of interest. The “worksite of interest” can be a new job or changes in exposures at an existing job due to the introduction of new processes or materials. “Concurrent asthma” or “coincident asthma” is asthma with onset while employed in the worksite of interest but not due to exposures in that worksite.

2. Asthma–work temporal relationship. It is necessary to document that the exacerbation of asthma was temporally associated with work, based either on self-reports of symptoms or medication use relative to work, or on more objective indicators like work-related patterns of serial measurements of the peak expiratory flow rate.

3. Conditions exist at work that can exacerbate asthma.

4. Asthma caused by work (i.e., occupational asthma) is unlikely

Estimation of WEA prevalence ranged from 13% to 58% in function of the different studies, with a median of 21.5% [275].

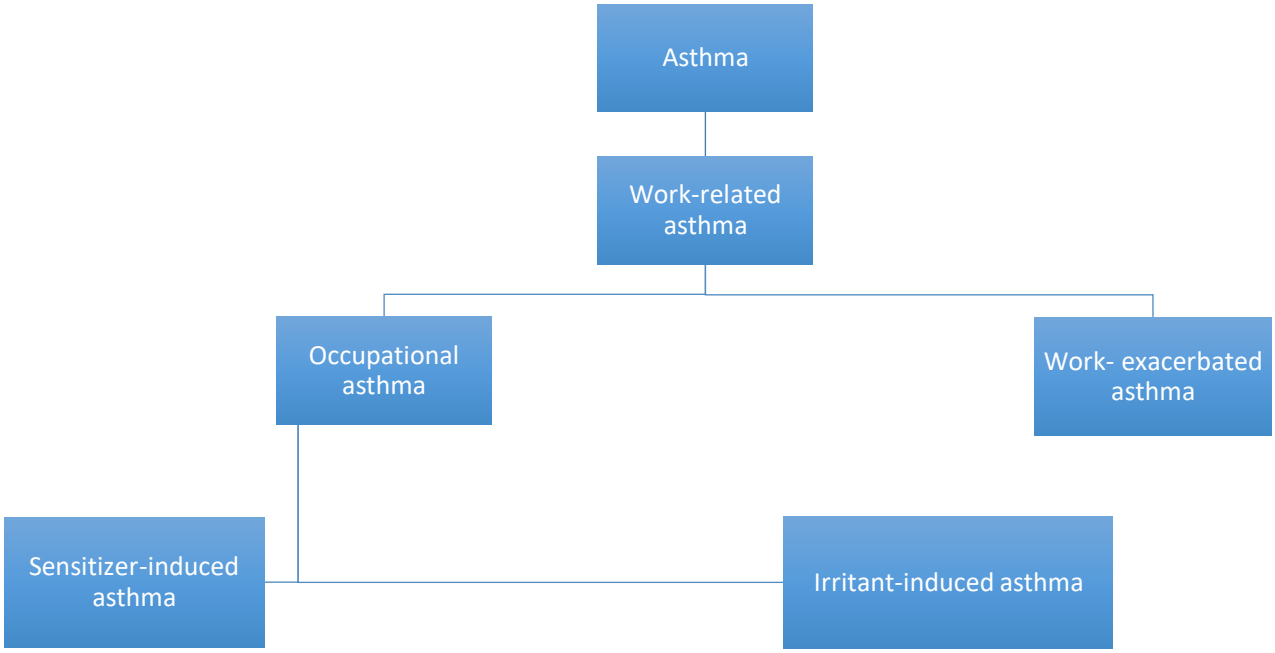

Figure 6 Classification of asthma in the workplace

OA encompasses sensitizer-induced asthma and irritant-induced asthma (Figure 6). Occupational asthma, is considered to represent 16% (95% CI, 10–22%) of cases of asthma

[19]. Its definition has be redefined by editorial consensus in the fifth edition of *Asthma in the workplace*: OA is a type of work-related asthma, that is caused by immunological (identified or presumed) (i.e sensitizer-induced asthma) and non-immunological (i.e irritant-induced asthma) stimuli present in the workplace [274]. Irritant-induced asthma is estimated to account for 5 to 15% of occupational asthma cases and sensitizer-induced up to 90% [276,277].

2.2 Sensitizer-induced asthma

2.2.1 Presentation of agents

Currently, more than 400 substances used at work have been documented as inducing OA (updated list of causal agents and occupations available at https://reptox.cnesst.gouv.qc.ca/en/occupational-asthma/Pages/occupational-asthma.aspx).

The main agents and work associated are displayed in table 3. The E-PHOCAS cohort gathers all sensitizer-induced asthma ascertained by specific inhalation challenge (SIC) between January 2006 and December 2018. The French national network of occupational health surveillance and prevention [Réseau National de Vigilance et de Prévention des Pathologies Professionnelles (RNV3P)] reported systematically all cases of probable or certain work related OA (including sensitizer induced OA and WEA) between 2001 and 2018 by completion of a standardized form in 32 centers realized by occupational physicians [278]. In both database, flour, isocyanate, persulfate, cleaning products represent the majority of the described cases. In RNVP3, was observed a decrease of cases for latex, wood, isocyanates and hairdresser products but an increase of cases for cleaning products and quaternary ammonium compounds between the first period (2001-2009) and the second (2010-2018). The work sectors the most represented were service activities (10.6%), food industry (10.2%) and healthcare activities (7.6%). There

is a lack of reports of recent trends the incidence of OA in the academic literature [279]. The incidence of OA appears to be declining based on physician- reporting or recognized compensation claims for the country with published data (-0.8 to -14.8 % annual change) even though few studies have adjusted appropriately for changes in the population at risk.

Table 3 Common Causative Agents in Sensitizer-Induced Occupational Asthma adapted from [269]

Agent	Workers at Risk of Exposure	Proportion of cases	
		In E-PHOCAS [280]	In RNV3P [278]
High-molecular-weight agents			
Animal allergens	Farmers, persons who work with laboratory animals, veterinarians	1.5	
Plants	Greenhouse workers, farmers	2.2	
Plant products (e.g., natural rubber latex)	Latex-glove makers and users, makers of other latex products	6.1	2.1
Cereals and grains	Farmers, grain workers, bakery workers	31.3	10
Other foods (e.g., milk powder and egg powder)	Food-production workers, cooks		
Fungi	Office workers, laboratory workers	0.4	
Enzymes	Laboratory workers, pharmaceutical workers, bakery workers	2.2	
Insects	Farmers, greenhouse workers	0.5	
Fish and crustaceans	Workers handling herring or snow crabs	0.7	
Vegetable gums (e.g., guar and acacia)	Printers, including carpet makers	0.2	
Low-molecular-weight agents			
Diisocyanates (e.g., toluene diisocyanate, hexa- methylene diisocyanate, and methylene diphenyl diisocyanate)	Makers of rigid or flexible polyurethane foam, installers of poly- urethane foam insulation, urethane spray painters, those who work with urethane adhesives or urethane molds in foundries	17.4	5.1
Acid anhydrides (e.g., phthalic anhydride, maleic anhydride, and trimellitic anhydride)	Makers of epoxy resins for plastics	2.4	3.5
Acrylic monomers	Chemical-industry workers, dental workers, aestheticians applying artificial nails	3.0	2.1
Wood dusts (e.g., from red cedar and exotic woods)*	Carpenters, sawmill workers, forestry workers	3.0	2.3
Complex platinum salts	Refinery workers, jewelry workers	1.2	
Other metal salts (e.g., nickel chromium)	Metal-plating workers, welders of stainless steel	3.6	
Biocides (e.g., glutaraldehyde and chlorhexidine)	Health care workers	1.4	4.8
Phenol-formaldehyde resin	Makers of wood products, foundry workers	1.3	3.5
Persulfates and henna	Hairdressers	6.6	6.5
Drugs (e.g., antibiotics)	Pharmaceutical workers, pharmacists	1.4	
Aliphatic amines (e.g., ethylenediamines and ethanolamines)	Lacquer handlers, soldering workers, spray painters, professional cleaners	0.8	2.8
Quaternary Ammonium compounds	Cleaning and disinfection of surfaces, instruments, and equipment, in healthcare and food processing facilities	3.2	5.3

2.2.2 High molecular weight vs. Low molecular weight: an empirical classification

Sensitizer-induced asthma is caused by immunological (identified or presumed) stimuli present in the workplace [274]. Historically, the first modern description of sensitizer-induced asthma has been made by Figley et al. in 1928 [281]. They described 32 patients from East Toledo, living less than a mile from an oil mill, who complained of asthma attacks. They had no asthma before moving to this district and reported that the onset of the attacks occurred from one to seventeen years after moving to the neighborhood. Attacks were most frequent and most severe during the fall and spring months when the wind attained its highest velocity, and these patients invariably had attacks when the wind blew toward their homes from the direction of the mill. Allergic scratch cutaneous test to castor bean confirmed a sensitization in these patients. The presence of a delay between the exposition and the symptoms suggested a sensitization process. For the records, sensitizer-induced asthma was used to be called *"occupational asthma with a latency period"* [274]. The first description of OA due to a chemical product, diisocyanate, was made in 1951 by Fuchs and Valade [29,282]. Originally the diagnosis of OA was mainly based on clinical history. The advent of SIC in the early 70's, developed by Pepys, has facilitated the identification of novel causal agents [283]. SIC consists in testing the controlled exposure of a patient, under laboratory conditions, to an agent encountered in their workplace [284]. An individual with sensitizer-induced asthma will manifest an asthma reaction subsequent to workplace exposure to a non-toxic dosage. In 1986 Chan-Yeung and coworkers have introduced a classification of sensitizer-induced asthma in function of the molecular weight of the agents [31]. Since, OA has traditionally been classified into two categories: high-molecular-weight (HMW) and low-molecular-weight (LMW) sensitizers. HMW agents are glycoproteins of vegetal, animal, and microbiological origin,

whereas LMW agents include reactive chemicals, transition metals, drugs, and wood dust. This historical classification implies that these two categories of agents are associated with distinct clinical phenotypes and pathophysiological mechanisms [285]. HMW sensitized asthma is supposed to be secondary to IgE mediated mechanisms, mechanism of LMW induced asthma mechanisms have not been fully elucidated. The main hypothesis for LMW asthma are IgE sensitization via hapten mechanism (LMW molecule becoming an antigen after binding to albumin protein), IgG mechanism or autoimmune mechanism [286]. The proposed molecular weights threshold differentiating HMW from LMW agents have ranged from 1 kDa to 20 kDa [287]. It has been suggested that 5 kDa would be the best cut off as the lowest molecular weight of allergen involve in OA is 6 kDa (natural rubber latex) [287].

Phenotypic differences between HMW and LMW asthma have been described in many occurrences, and were summarized in a recent literature review [287] (table 4). Asthma induced by HMW agent exhibit more often rhinoconjonctivitis compared to LMW agents. Also, rhinoconjonctivitis more often precedes the onset of asthma. On the other hand, compared to HMW agents, LMW agents elicits more often chest tightness, sputum at work and provoke more exacerbations. Nevertheless, contradictory result has being found concerning comparison of severity, or functional outcomes in both HMW and LMW.

Table 4 Clinical and functional differences of phenotypes based on agents' molecular weight, adapted from [287]

Characteristics	**High molecular weight agents**	**Low molecular weight agents**
Associated work-related disorders:		
Rhinoconjunctivitis	● Higher prevalence [280,288–292] ● More intense symptoms [292,293] ● More often precedes the onset of asthma [288,292,293]	● Higher prevalence compared to other LMW agents: acrylates [294], platinum salts, [295]
Urticaria	● More frequent but not significant in multivariate regressions [280]	
Contact dermatitis		● More frequent [296] ● No difference between HMW and LMW agents [280]
Asthma-related outcomes:		
Latency period before onset of symptoms		● Median/mean latency period: - Shorter for Western red Cedar compared to HMW agents and isocyanates [297] - Longer for LMW agents [298] - No difference between HMW and LMW agents [249,280,290]
Asthma symptoms		● More often chest tightness at work [280] ● More frequent daily sputum at work [280]
Asthma severity	● Daily dose of inhaled corticosteroids: No difference between HMW and LMW agents [249,280] ● Severe asthma (ERS/ATS definition) ‡: No difference between HMW and LMW agents [299]	● Moderate-severe asthma (i.e. FEV1 <70% predicted or C PD_{20} methacholine ≤300 µg): No difference between HMW and LMW agents [300] ● Moderate-severe persistent asthma (GINA classification): Higher risk with LMW agents [290]
Asthma control		● Exacerbations: More frequent with LMW agents [280]
Baseline airway obstruction	● More marked [280] ● No difference between HMW and LMW agents [249,290,299,301]	
Baseline NSBH	● Lower degree of NSBH [302,303] ● No difference between HMW and LMW agents [249,290,299,301]	
Outcome of asthma after avoidance of exposure	More frequent persistence of NSBH after cessation of exposure [304–306] but no difference in the rate of symptom recovery [306] ● No effect on time trend in NSBH recovery [307]	

Both environmental and host risk factor may differ in HMW and LMW induced asthma. The level of airborne exposure have been associated with an increased risk of developing OA subsequently to HMW or LMW exposure [308]. Exposure to cigarette smoke was associated with an increased risk to develop OA among worker exposed to HMW agents while such an increased risk of OA has not been documented for LMW agents [287,309,310]. Atopy, defined as a positive skin prick test or the presence of sIgE to at least one ubiquitous inhalant allergen, is a major risk factor of the development of IgE-mediated OA among exposed worker [287,308]. The positive predictive value of atopy in predicting the risk of occupational asthma to HMW agents is approximately 30% [308,311]. This value has been deemed insufficient to justify the eviction of atopic individuals from workplaces where they are exposed to potential triggers [308,312]. On the other hand, the scientific literature does not support association between atopy and risk of OA caused by LMW agents [287].

The diagnostic strategy for sensitizer-induced asthma varies depending on the category of the agent in question. Diagnose sensitizer-induced asthma may be difficult. A stepwise algorithm resuming the strategy is exposed in figure 7. Briefly, after baseline assessment on clinical and occupational history, NSBH assessment and immunologic testing are performed. The absence of NSBH if the subject is exposed at work can rule out OA. A highly positive immunologic test can confirm OA. Otherwise, if the subject is still working, an assessment of functional and inflammatory changes in work and off work can be made. If the patient is not working anymore a SIC can be performed if available. In the most prevalent LMW agents, such as isocyanate and persulfates, no immunologic testing are available. Furthermore, meta-analysis estimates indicate that among LMW agents, when sIgE measurements are available, sIgE measurements demonstrate a low sensitivity (19-40%) for diagnosing OA (Lux et al., 2019). On the contrary, the same meta-analysis concluded that sIgE testing reached a high sensitivity (74-81%) for HMW agents (Lux et al., 2019).

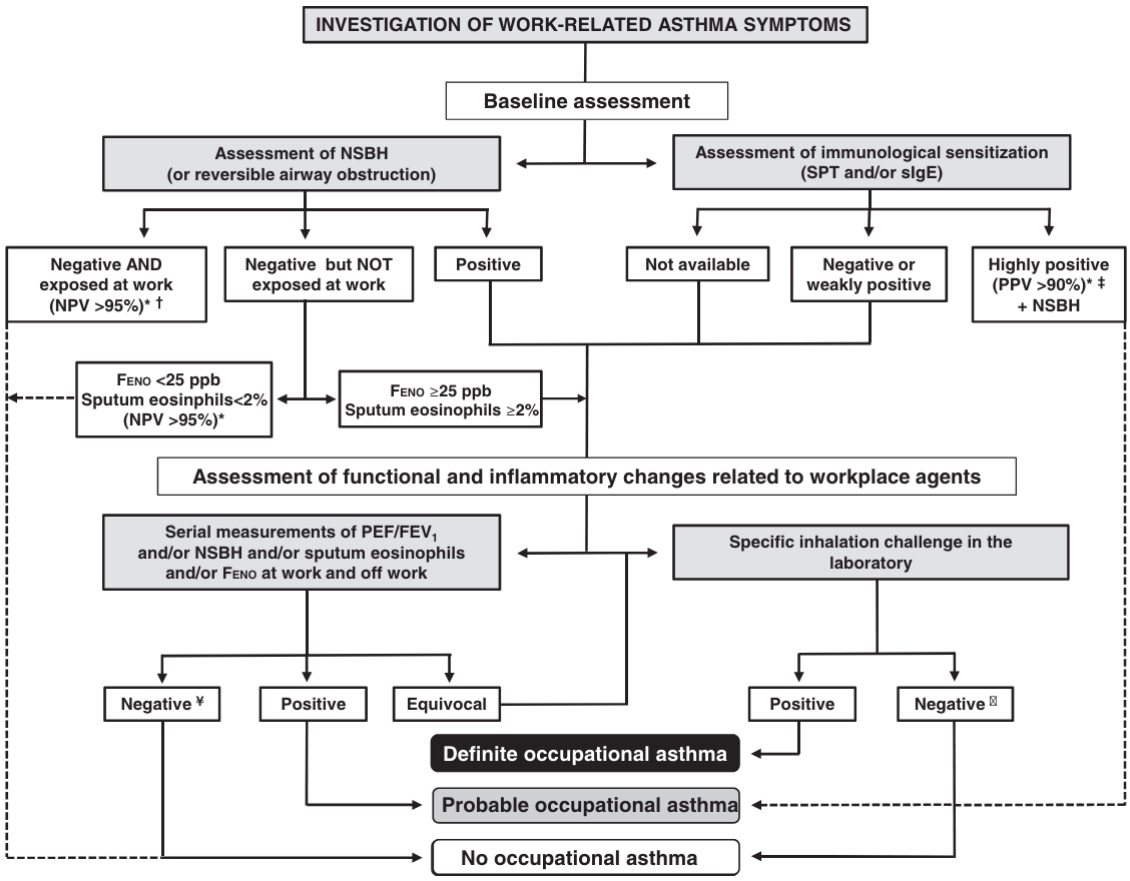

Figure 7 Stepwise algorithm for diagnosing OA, from [313]

*High NPV and PPV are applicable only to selected populations of subjects with a high pretest probability of OA (ie, tertiary centers). †Consider further investigation at the workplace if the clinical history is highly suggestive of OA because the absence of NSBH has been documented even after an asthmatic reaction induced by occupational agents. ‡ In subjects with NSBH when immunologic tests have been validated against SIC. Increasing the cutoff value for a positive sIgE test result of greater than or equal to 2.22 kUA/L for wheat flour, greater than or equal to 9.64 kUA/L for rye flour, and greater than or equal to 4.41 kUA/L for latex provides a PPV for a positive SIC result higher than 95%. ¥Consider an SIC in the laboratory if the clinical history is highly suggestive of OA. #Consider a workplace inhalation challenge or serial PEF recording at work if the clinical history is highly suggestive of OA.

Concerning the management, differences between HMW and LMW induced asthma are scarce. Once the diagnosis of OA is confirmed, the best way to improve the outcome is to remove patient from the exposure [314]. Indeed, Henneberger and coworkers conducted a systematic review of workplace intervention for treating occupational asthma [315]. The review is based on 26 non randomized studies that included 1,695 participants. The quality of evidence was assessed to be very low because of a risk of bias of selection, a high heterogeneity of the study and likelihood of publication bias. Nevertheless, they concluded that removed exposure may

improve both asthma symptoms and lung function, while reduced exposure may improve asthma symptoms but not lung function. Additionally treatment of OA should follow GINA asthma guidelines [13] with regard to asthma education, control of exposure to environmental triggers and appropriate pharmacotherapy. Associated comorbidities, such as occupational rhinitis [316], vocal cord dysfunction [317] and post-traumatic stress disorders [318] should be carefully investigated.

For HMW induced asthma, treatment by allergen specific immunotherapy have been studied [319]. All published randomized double-blind trials of desensitization in occupational allergy concern latex allergy [320–323], with the exception of one involving wheat flour allergy [324] (80). In general, sublingual desensitization was better tolerated than subcutaneous desensitization, with encouraging results in term of improvement of symptoms despite the limited numbers involved (maximum 40 patients). In the absence of a marketed extract, it has not been used in clinical practice.

In sensitizer-induced asthma, the molecular weight of the agent plays a role in the outcome after the agent removal. A large meta-analysis gathering 1681 patients, estimated that after the agent removal, only 32% (26-38%) of patients experienced complete symptomatic recovery [306]. Even though complete symptomatic recovery at follow up was not different in subjects with HMW and LMW induced asthma, HMW-induced asthma was associated with a higher persistence of NSBH at follow up compared to LMW-induced asthma.

2.2.2 Limitations of the classification of sensitizer-induced asthma according to the molecular weight of the agent

Available data on inflammatory phenotypes in sensitizer-induced OA did not show major difference depending on the nature of the offending agent. Boulet and colleagues observed comparable pathological characteristics in bronchial biopsies obtained from subjects with OA resulting from HMW and LMW agents [325]. Other bronchial biopsy studies of subjects with LMW induced OA did not found different inflammatory changes in comparison to allergic asthma [326–328]. Moreover, demonstration has been made that eosinophil inflammation, measured by induced sputum, is increased by the exposure of the offending agent independently of its molecular weight [249,329]. Interestingly, Lemiere and coworkers described in a cluster study on 98 sensitizer-induced asthma an association between an increase of FeNO during the SIC with exposure to HMW agents [330]. This result was confirm in a subsequent study on 1179 sensitizer-induced asthma [280].

There is accumulative evidence that OA due to some LMW agents shares phenotypic characteristics with OA caused by HMW agents. Doyen and coworkers refer to them by the label “atypical LMW agents” [287]. First, for some LMW such as platinum salt, acid anhydrides, reactive dyes and chloramine-T, the presence of specific-IgE has been documented in subjects with OA [287,295]. Secondly, for OA caused by acrylate, no specific-IgE have been documented but phenotypic traits usually attributed to HMW associated OA have been observed such as a high rate of rhinitis and greater post-exposure increase in FeNO [287,294,330].

To summarize, sensitizer induced asthma is the most frequent type of OA, elicited by immunological mechanisms. It can be caused by various number of agents. Classification of sensitizer-induced asthma has been made empirically on the molecular weight of the offending

agent. This latter has been shown to distinguish patients presenting different clinical forms, diagnostic biomarker, and risk factors. HMW induced asthma is driven by an IgE mediated mechanism, whereas LMW agent induce asthma through non elucidated mechanism. Nevertheless, the relationship between inflammation patterns and sensitizer induced asthma has not been thoroughly investigated. In addition, asthma induced by LMW agents seems to be a heterogen entity with atypic LMW agents such as acrylate or platinum salt.

2.3 Irritant-induced asthma

2.3.1 Acute and subacute irritant-induced asthma

Irritant-induced asthma is caused by non-immunological stimuli present in the workplace [274]. The first description of irritant-induced asthma has been made by Brooks and colleagues in 1985 [30]. They described ten individuals without preexisting respiratory condition who developed asthma symptoms following a single exposure to high levels of an irritating vapor, fume, or smoke. All subjects exhibited NSBH. This phenomenon has been labelled reactive airway disorder syndrome by the authors. The reaction occurring within 24 hours after exposure, a non-immunologic mechanism reaction was suggested. This reaction also used to be called *"occupational asthma without a latency period"* [331]. Subsequently, reports have been made of new onset of asthma after an acute exposure that did not cause very severe respiratory symptoms. These new onset asthma cases can even develop insidiously over a few days to months after the massive exposure, as it was described for firefighters at the world trade center in 2001 [332].

In 2014, a EAACI task force proposed to redefined the labels of irritant-induced asthma in function of the mode of exposure, the onset of asthma in order to estimate the diagnostic likelihood for each individual [331]. Definite irritant-induced asthma relates to acute-onset

irritant-induced asthma manifested by the rapid onset of asthma within 24 hours of a single, very high-intensity exposure to an irritant compound. Probable irritant-induced asthma is the development of asthma after multiple symptomatic moderate-to high-level exposures to irritants, also called subacute irritant-induced asthma (figure 8).

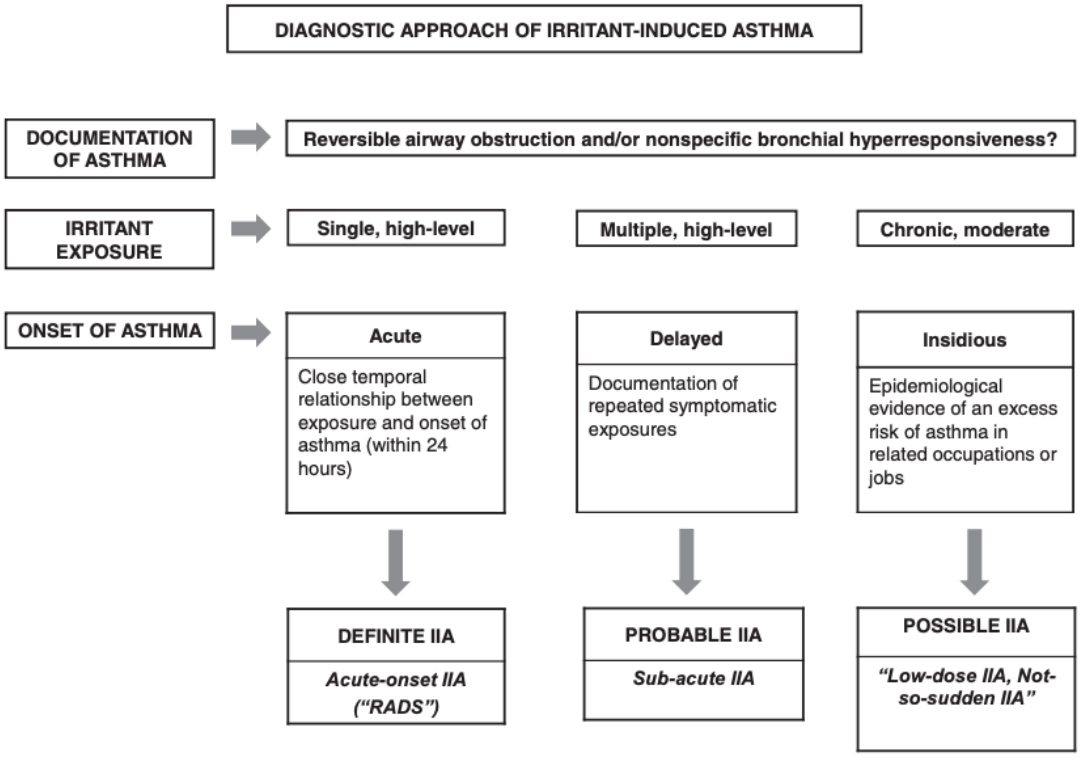

Figure 8 Diagnostic approach for irritant-induced asthma. From [277]
RADS, reactive airways dysfunction syndrome.

Numerous irritant agents illustrated in table 5 have been observed to provoke acute and subacute irritant-induced asthma. Two suspected pathophysiologic mechanisms, different but interlinked are evoked.

- Tissue irritation describes epithelial cell damage caused by the exposure, releasing inflammatory cells such as neutrophils, lymphocytes, eosinophils and macrophages into the airways hyperresponsiveness and airway remodeling [277,333,334]. In animal

models, chlorine exposure has been shown to induce the production of both reactive oxygen species and cysteinyl leukotrienes [277,334].

- Sensory irritation correspond to a neurogenic inflammation caused by irritant exposure, inducing release of neuropeptide, substance P and neurokinin which trigger responses from immune, vascular and smooth muscle cells via specific receptors [286]. A single neuronal receptor, transient receptor potential cation channel subfamily A member 1 (TRPA1), can recognize a wide variety of occupational allergens such as isocyanates, and other environmental irritants. Animal models of chemical induced airway hyperresponsiveness, independent of T and B cells, have been proven to be dependent to TRPA1 stimulation and mast cells activation [335,336]. It has been concluded that neuro-immune interactions, involving TRPA1, TRPV1 and mast cells, are crucial in developing nonatopic irritant-induced airway hyperresponsiveness, even in absence of cellular manifestations of inflammation.

Table 5 Examples of exposures involved in acute and subacute irritant-induced asthma from [277]

Chemical category	Example of chemicals
Inorganic gases	Chlorine (eg, pulp mills or released by mixing sodium hypochlorite with acids), chloramines released by mixing sodium hypochlorite with ammonia, sulfur dioxide, nitrogen oxides, ozone
Inorganic acids	(Per)acetic (disinfectant), sulfuric, hydrochloric, hydrofluoric (thermal degradation product of fluorinated hydrocarbons), and hydrobromic acids
Inorganic alkali	Ammonia, sodium hydroxide, hydrazine
Inorganic dusts	Calcium oxide (lime) and cement (eg, World Trade Center dust)
Halogenated	Bromochlorodifluoromethane (fire extinguisher), heated fluorinated hydrocarbons (cooling agents; thermal degradation into hydrofluoric acid), orthochlorobenzylidene malononitrile (tear gas)
Solvents	Perchloroethylene
Fumes	Diesel exhaust, fire smoke, paint and urea fumes, fumes of iodine and aluminium iodide, dimethylaminoethanol (corrosion inhibitor)
Mixtures of chemicals	Cleaning agents (bleach, ammonia, detergents, degreasing sprays, decalcifiers, disinfectants)
Potential respiratory sensitizers	Isocyanates (eg, thermal degradation of polyurethane insulation materials), aldehydes, phthalic anhydride

Phenotypic description of acute and subacute irritant-induced asthma are scarce. Lantto and colleagues both studied short and long term prognosis of acute and subacute irritant-induced asthma [337,338]. irritant-induced asthma patients exhibited poorer asthma control, higher rate of high-level treatment, more exacerbation, lower FeNO level and lower atopy compared to sensitizer-induced asthma. Long-term prognosis may also be worse in irritant-induced asthma than sensitiser-induced asthma in terms of asthma control. In a 6-year follow-up study after diagnosis, 56% of irritant-induced asthma patients presented with uncontrolled asthma compared to 30% for those with LMW-induced asthma, but there were no significant differences in exacerbation rates [337]. Here again, FeNO levels were significantly lower in irritant-induced asthma (median value of 14 vs. 23 ppm for LMW induced asthma).

2.3.2 Low dose or chronic exposure irritant-induced asthma

Low dose irritant-induced asthma or possible irritant-induced asthma is the delayed development of asthma after chronic or repeated exposure to low-to-moderate levels of irritants substances (figure 8) [277]. It has been suggested stop using the term "low" or "moderate" dose and prefer "chronic exposure" as, in most cases, levels of irritant exposure are unmeasured and associated with health hazards [339,340]. Chronic exposure irritant-induced asthma have been investigated in epidemiological studies. In a large cross-sectional study among 340155 Estonian participants, the risk of current physician-diagnosed asthma was increased among worker with lifetime exposure to low level of irritant estimated by an asthma specific job exposure matrix [341]. More recently, in 4469 adults from the Nutrinet-Santé Cohort, irritant exposure was associated with both current adult onset asthma and uncontrolled adult onset asthma [342]. Increased risk of new onset asthma for exposed subjects have also been demonstrated in longitudinal setting. Notably in the RHINE study, gathering 13284 participants, exposure to cleaning products was associated with an increased risk of new onset asthma mostly in non-atopic subjects [343]. A meta-analysis including 21 studies evaluating the association between occupational cleaning exposure and asthma risk showed a pooled relative risk of 1.50 (95%CI 1.44 -1.56) [344].

Phenotypical descriptions of chronic irritant induced asthma are scarce. Andrianjafimasy et al. described in the EGEA study an association between occupational irritant exposures with a specific cluster of asthmatic patients identified by a cluster-based statistical method [345]. This cluster was characterized by adult-onset pattern, poor lung function, high blood neutrophil counts, and high fluorescent oxidation product level. In ECRHS, case-case analysis revealed that compared to non-exposed asthmatic subjects (office worker), asthmatic cleaners exhibited

less atopy, more chronic bronchitis and lower lung function [346]. In a cross-sectional study gathering 1008 subjects, Wang et al. described an association between both exposure to irritant peaks and industrial cleaning agents' exposure with non-atopic asthma but not with atopic asthma [347].

To summarize, irritant induced asthma can be induced by acute, subacute or chronic exposure to irritant products (figure 8, table 5). Two main non immunological mechanisms are suggested: tissue irritation and sensory irritation. No risk factor has been identified, and only limited information is available concerning its phenotypic characterization.

Definitions vary with time, according to the current status of evidence. Research on non-occupational asthma have outpaced those on occupational asthma. Key elements of the characterization of non-occupational asthma such as inflammatory pattern and comorbidities have not been thoroughly investigated in occupational asthma.

Third part:
Thesis objectives

As demonstrated in the introduction section, asthma classification has evolved last decades because the constatation have been made that the one-size-fits-all approach to treatment has shown limitations. The characterization of patients in term of triggers, clinical presentation and inflammatory pattern have brough new insights to deal with the heterogeneity of the disease. This thesis work is specifically devoted to improve the characterization of occupational asthma. The underlying question is: What are the relationships between clinical presentation, the offending agent and inflammatory pattern in occupational asthma (figure 9). Thus, the central hypothesis of this work is that phenotyping occupational asthma using trigger factor, clinical presentation and inflammatory pattern distinguishes distinct group of patients. To test this hypothesis, the PhD work overall aimed to provide better insights in occupational asthma phenotypes by:

1) Characterizing the clinical, functional, and inflammatory profiles of sensitizer-induced asthma caused by a specific agent, Quaternary Ammonium Compound (QAC)

2) Investigating the impact of the presence of comorbidity, in particular work-related dysphonia, in sensitizer-induced asthma

3) Investigating the clinical and functional characteristics of sensitizer-induced asthma according to induced sputum inflammatory patterns

4) Characterizing the association between irritant occupation exposure and specific-IgE sensitization patterns in adult-onset asthma

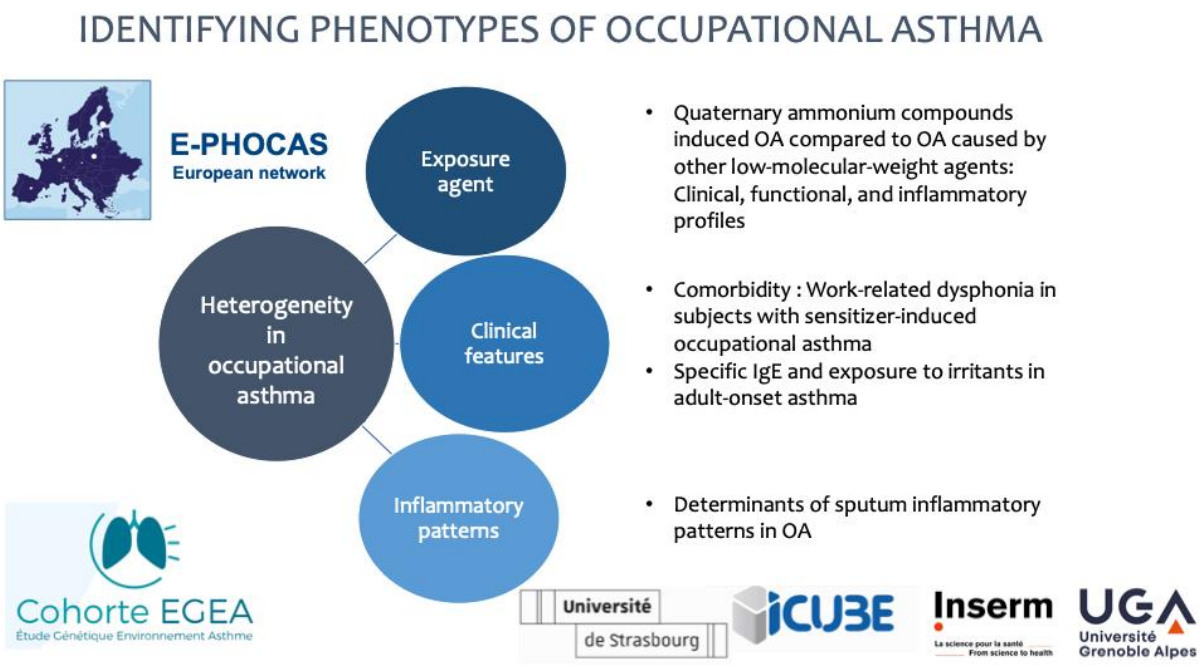

Figure 9 Occupational asthma: a heterogeneous disease

Fourth part: Methods

Approaches to study occupational asthma

The first approach to study OA consists in investigating clinically confirmed cases of occupational asthma, defined using specific tests to prove the causality of exposure to a specific agent in the disease. This approach, allowing well established causality at the individual level, has been commonly used for the description of sensitizer-induced asthma. However, for chronic irritant-induced asthma, the causality cannot be ascertained at an individual level. The second approach is to use epidemiological studies to investigate the statistical association between occupational exposure and adult-onset asthma. Following this approach, the causality, that cannot be proven at the individual level as in the first approach, has to be assessed and discussed at the populational level according specific criterions [348].

This thesis work is based on both approaches, including the E-PHOCAS cohort representing the first one and the EGEA cohort the second.

4.1 E-PHOCAS Cohort

4.1.1 Presentation of the cohort

The European network on Phenotyping of Occupational Asthma (E-PHOCAS) cohort includes all consecutive subjects who showed a positive SIC between January 2006 and December 2018 in 20 specialized centers from 11 European countries (Figure 10).

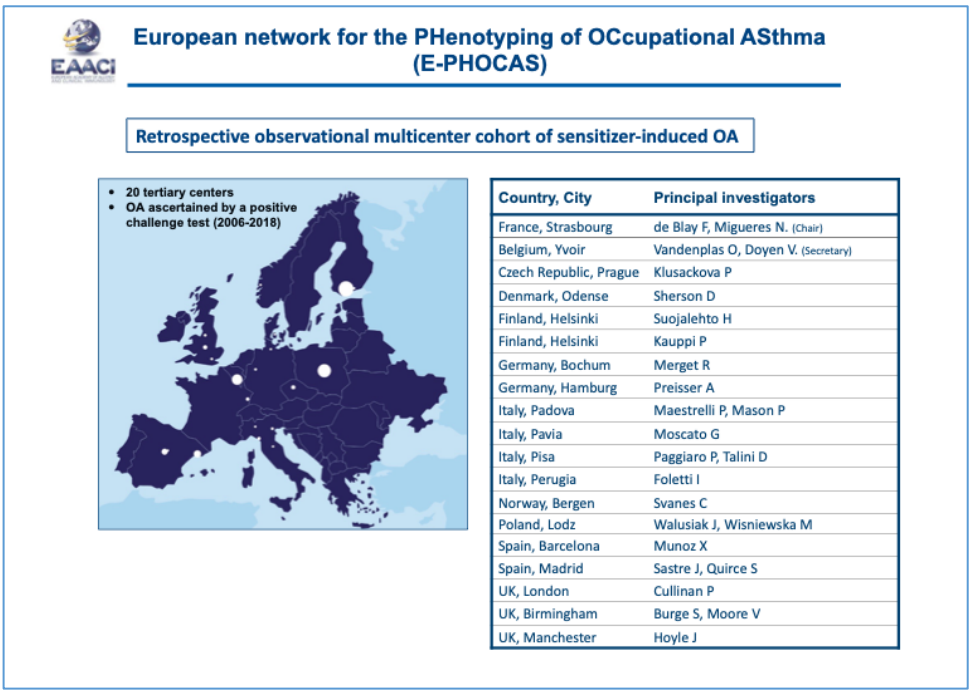

Figure 10 E-PHOCAS cohort centers

The overall cohort gather 1180 sensitizer induced asthmatic patients. For the purpose of this PhD work, subjects were selected in function of detailed information on asthma outcome, baseline induced sputum information and pre-and-post SIC induced sputum data figure 11.

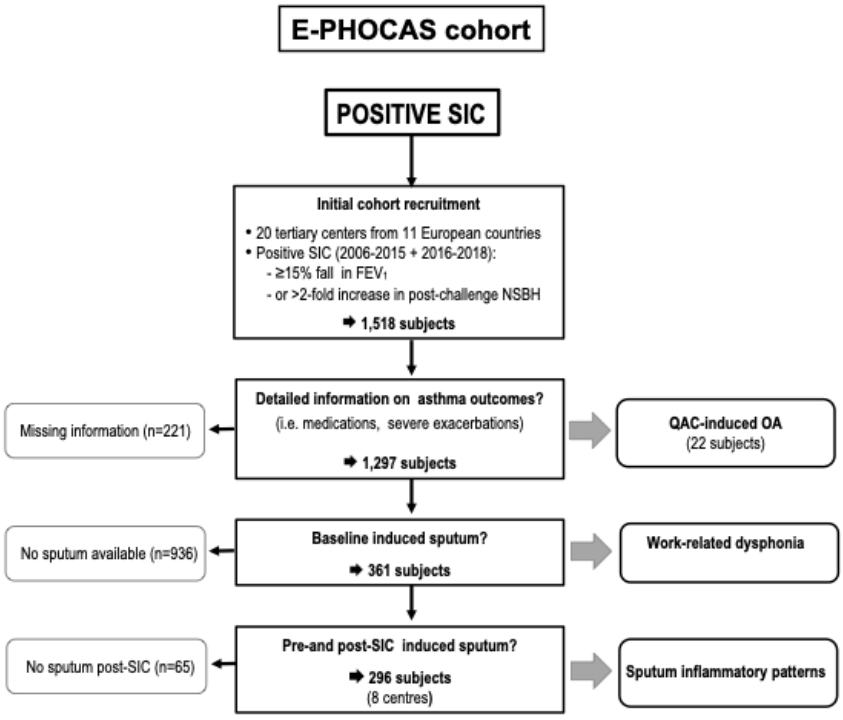

Figure 11 E-PHOCAS cohort flow chart

4.1.2 Collected data

The participating investigators were asked to enter in the database the information collected at the time of the diagnostic investigation of subjects with a positive SIC. Data pertaining to respiratory symptoms were not collected through a standardized questionnaire. The requested information included: 1) causal agent and job; 2) demographic characteristics; 3) clinical features (smoking habits, atopic status [defined by at least one positive skin -prick test to a battery of common aeroallergens]); 4) nature and timing of work -related respiratory symptoms and exposure; 5) associated disorders (physician -based diagnosis of work -related rhinitis, conjunctivitis; contact urticaria, and/or dermatitis, and sinusitis); and 6) detailed asthma medications used during the last month of exposure at work .

Since most of the participating centers failed to use validated instruments for the assessment of asthma control throughout the study period, “poor symptom control” was defined by the need for an inhaled short-acting β_2-agonist (SABA) once or more a day as proposed in the recommendations of the American Thoracic Society (ATS) issued in 2000 [349]. Severe asthma exacerbations were defined as those requiring oral corticosteroids for at least three consecutive days or an emergency room visit or a hospitalization [350].

The definition of severe asthma was adapted from the European Respiratory Society/American Thoracic Society criteria [350] and required a high-level treatment according to the Global Initiative for Asthma (GINA) [351] (i.e., treatment step 4-5 including a high dose of inhaled corticosteroid [ICS] and a second controller or systemic corticosteroid use >50% of the previous year) together with any one of the following criteria indicating uncontrolled asthma: 1) “poor symptom control”; 2) two or more severe exacerbations in the previous year; or 3) airflow obstruction defined by a forced expiratory volume in 1 sec (FEV1) <80% predicted value together with a FEV1/forced vital capacity (FVC) ratio <0.70 [299].

4.1.3 Specific inhalation challenges

Specific inhalation challenge (SIC) aims to investigate empirically the specific reactivity of the airways to occupational agents. European recommendations to harmonize practice have been published by the European Respiratory Society [284]. SIC is considered to be the gold standard for the diagnosis of sensitizer-induced occupational asthma. However, for safety reasons, it can only be performed in referral centers and requires special equipment.

The test procedure is summarized in Figure 12. A SIC s typically conducted over a three-day period in a hospital setting. It is recommended that treatment with inhaled corticosteroids be

discontinued at least 72 hours prior to the test, if feasible. A positive SIC result is defined by either a ≥15% fall in FEV1 at any time during the post-challenge monitoring or a twofold or greater increase in the post-challenge level of NSBH (i.e., a pre/post $PC/PD_{15-20\%}$ ratio ≥2) in the absence of a ≥15% fall in FEV1 [284].

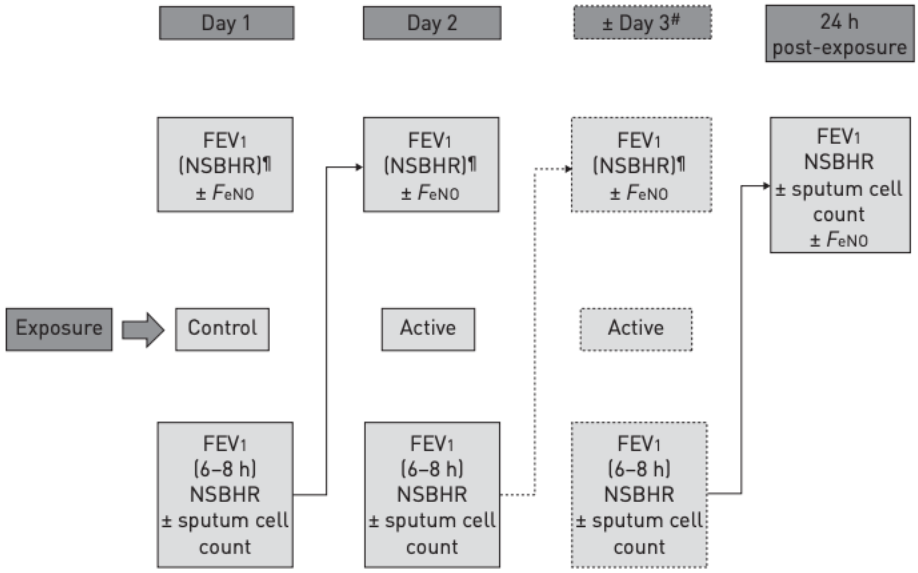

Figure 12 Schematic flowchart for performing specific inhalation challenge with an occupational agent from [284]

The timing of some measurements varies between centres. FEV1: forced expiratory volume in 1 s; NSBHR: nonspecific bronchial hyperresponsiveness; FeNO: exhaled nitric oxide fraction. # : proceed to additional active challenge(s) when the changes in FEV1 on day 2 are equivocal or negative and challenge with a higher dose is considered appropriate, or when there is a significant increase in sputum eosinophils or in FeNO post-day 2 challenge. ¶: NSBHR can be measured in the morning before the control and active challenge exposures provided that no inhaled bronchodilator is administered.

On the first day, a single-blind placebo exposure is conducted, with the duration increasing incrementally (10 seconds, 1 minute, 5 minutes, 10 minutes, 30 minutes and 60 minutes). If possible, the placebo used should have the same appearance as the sensitizing agent. The most commonly used substances are, for example, lactose powder for a flour test, or vinyl gloves for a latex test. Clinical monitoring is conducted for a period of six to eight hours, with FEV1 monitored via spirometry. A decrease of more than 10% in FEV1 on exposure to placebo indicates that the test should not be carried out, given the hypothesis of a non-specific reaction

to an irritant product. On the second day, the patient is exposed to the suspect agent, with the same clinical and spirometric monitoring protocol as that employed on the preceding day.

In order to evaluate the compliance with international recommendations on SIC with occupational agents [284] , the investigators completed a questionnaire on the following items prior to participating in the E-PHOCAS cohort: 1) absence of respiratory tract infection or asthma exacerbation within the previous 4 weeks; 2) duration of ICS withdrawal before the SIC procedure; 3) performance of a control (placebo) test on a separate day before challenging the subjects with occupational agents; 4) lower limit of FEV1 value considered a contra-indication for performing a SIC procedure 5) method used for delivering challenge exposures with workplace agents (i.e., "realistic" challenge or inhalation of an "allergen extract"; and 6) functional monitoring of at least 6 hours after the end of challenge exposure.

All participating centers conformed with safety and reliability requirements. The lower limit of FEV1 was 70% of the predicted value in three centers, 65% in one center; 60% in four centers. In all centers, ICS were withheld 2 or 3 days before the SIC procedure.

The database collected information on the maximum fall in FEV1 expressed as percent from baseline value that was recorded during: 1) the period between the end of the challenge exposure and the 60^{th} minute post-exposure (i.e., the "early component" of the bronchial response) and 2) the period between the 60^{th} minute post-challenge and the end of the post-SIC follow-up (i.e., the "late component" of the bronchial response). The results of the SICs were interpreted *a posteriori* according to standardized criteria.

Based on the presence of an immediate and/or a late asthmatic component, the pattern of the FEV1 bronchial response was categorized as an "isolated immediate", "isolated late", or "dual"

reaction. In this analysis, we compared isolated immediate reactions with late-component reactions, including isolated late and dual responses.

4.1.4 Assessment of nonspecific bronchial hyperresponsiveness

The level of nonspecific bronchial hyperresponsiveness (NSBH) was expressed as the concentration or dose of the pharmacological agent inducing a 20 % fall in FEV1 ($PC/PD_{20\%}$) according to the bronchoprovocation method used in each center. These $PC/PD_{20\%}$ values were used to calculate pre/post-BPT ratios. Since participating centers used four different methods, the level baseline NSBH was only categorized as “absent”, “mild”, or “moderate-to-severe” based on available recommendations [352–354] or a consensus Delphi approach among investigators. The bronchoprovocation methods and threshold values used for defining the level of baseline NSBH are detailed in Table 6.

Table 6 Methods for measuring nonspecific bronchial hyperresponsiveness

Method (pharmacological agent)	**No. of centers**	**Threshold values for nonspecific bronchial hyperresponsiveness**		
		Moderate-to-severe	**Mild**	**Absent**
Tidal breath method (histamine/methacholine)[352,354]	2	PC_{20} <1 mg/ml	PC_{20} :1-16 mg/ml	PC_{20} >16 mg/ml
Five-breath dosimeter method (methacholine) [352,354]	3	PD_{20} <0.1 mg PC_{20} <1 mg/ml	PD_{20}: 0.1-1.5 mg PC_{20}:1-16 mg/ml	PD_{20} >1.5 mg PC_{20} >16 mg/ml
Rapid dosimeter method (histamine) [353]	1	PD_{15} <0.4 mg	PD_{15} : 0.4-1.6 mg	PD_{15} >1.6 mg
Reservoir bag dosimeter method (methacholine) [355]	1	PD_{20} or PD_{100} sRt <0.1 mg	PD_{20} or PD_{100} sRt: 0.1-0.3 mg	PD_{20} or PD_{100} sRt >0.3 mg

Legend: *FEV1*, forced expiratory volume in 1 second; *NSBH*, nonspecific bronchial hyperresponsiveness *PC/PD$_{15-20}$*, provocative concentration/dose of pharmacological agent inducing a 15-20% fall in FEV1; *PD$_{100}$ sRt*: provocative concentration of pharmacological agent inducing a doubling of specific airway resistance (sRt).

4.1.5 Sputum Induction and Processing

The eight centers who performed sputum induction completed a detailed questionnaire pertaining to the method used for the induction and analysis of sputum samples. Sputum was induced through different methods, including the inhalation of nebulized isotonic saline (n=1), a single concentration of hypertonic solutions (i.e., 3%; n=1) or increasing concentrations of hypertonic solutions ranging (i.e., 3%, 4%, and 5%; n=7) for a maximum cumulative duration of 15 to 40 minutes [221]. The processing of sputum samples was carried out either by selecting viscid portions from the expectorate (3 centers) [356] or using the whole expectorate (5 centers)

[357]. Homogenization of the sample was achieved by adding dithiothreitol (0.1%). All centers applied quality criteria based on the cell viability (i.e. at least 40%) and the level of contamination by squamous cells [221]. The accepted squamous cell contamination was <20% in five centers, <30% in one center, and <50% in two centers. The differential cell count was determined by counting a minimum of 400 nonsquamous cells. Sputum eosinophil and neutrophil counts collected at baseline and 24 hours after the challenge exposure were expressed as a percentage of nonsquamous cells. Available information indicates that using viscid portions from the expectorate or the whole expectorate as well as different nebulizers and saline concentrations does not significantly affect differential sputum cell counts [221,358].

4.1.6 Ethics

Approval for this retrospective analysis of anonymized data was obtained from each local Institutional Review Board. The central database at the Strasbourg University was approved by the "*Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé*" and the "*Commission Nationale de l'Informatique et des Libertés*".

4.1.7 Strategy of analysis and statistical tool

In order to identify phenotypes of sensitizer induced asthma using trigger factor, comorbidity and inflammatory pattern patients were compared in function of the outcome (QAC induced asthma, work-related dysphonia and induced sputum inflammatory profiles). Comparisons were performed between groups using the Fischer's exact test or chi-squared test for categorial variables and nonparametric tests for numeric variables.

Multivariable logistic regression analyses were conducted in order to identify the characteristics associated with the outcome using a binomial generalized linear model. The

most parsimonious models were selected using a stepwise procedure based on the Akaike information criterion. The independent variables incorporated in this model included both *a priori* variables (for example age gender and smoking status) and variables with a *P*-value ≤0.1 in univariate comparisons.

Concerning QAC induced asthma, a multivariable linear regression model was conducted in order to assess the association between sensitization to QAC with the magnitude of post SIC change in sputum eosinophils using the same procedure to select the most parsimonious model. Missing values were not imputed.

4.2 EGEA Cohort

4.2.1 Presentation of the cohort

The EGEA (Etude Epidémiologique des facteurs Génétiques et Environnementaux de l'Asthme) [https://cohorte-egea.fr] epidemiological cohort was initiated in 1991 to meet the following general objectives:

- Identify genetic factors in asthma
- Identify environmental factors in asthma
- Clarify the clinical heterogeneity of asthma, i.e. the different forms in which this disease manifests itself.

The EGEA cohort was set up in 5 French cities: Paris, Lyon, Grenoble, Montpellier and Marseille.

To meet the research objectives, 388 children and adults with asthma were recruited from pneumology or pediatrics consultations in one of the 8 hospitals participating in the study (Cochin, Lyon, Marseille, Montpellier, Necker, Bichat, Grenoble and Trousseau), then their first-degree relatives (parents and siblings for participants recruited as children, or spouses and children for participants recruited as parents) and 415 children and adults from the general population ("controls") were included. A total of 2047 participants were examined for the first time between 1992 and 1995. Around 12 years later (2003-2007), study participants were invited to take part in a first follow-up, and again around 7 years later (2011-2013). A new follow-up of the study is currently underway. The protocol is illustrated figure 13.

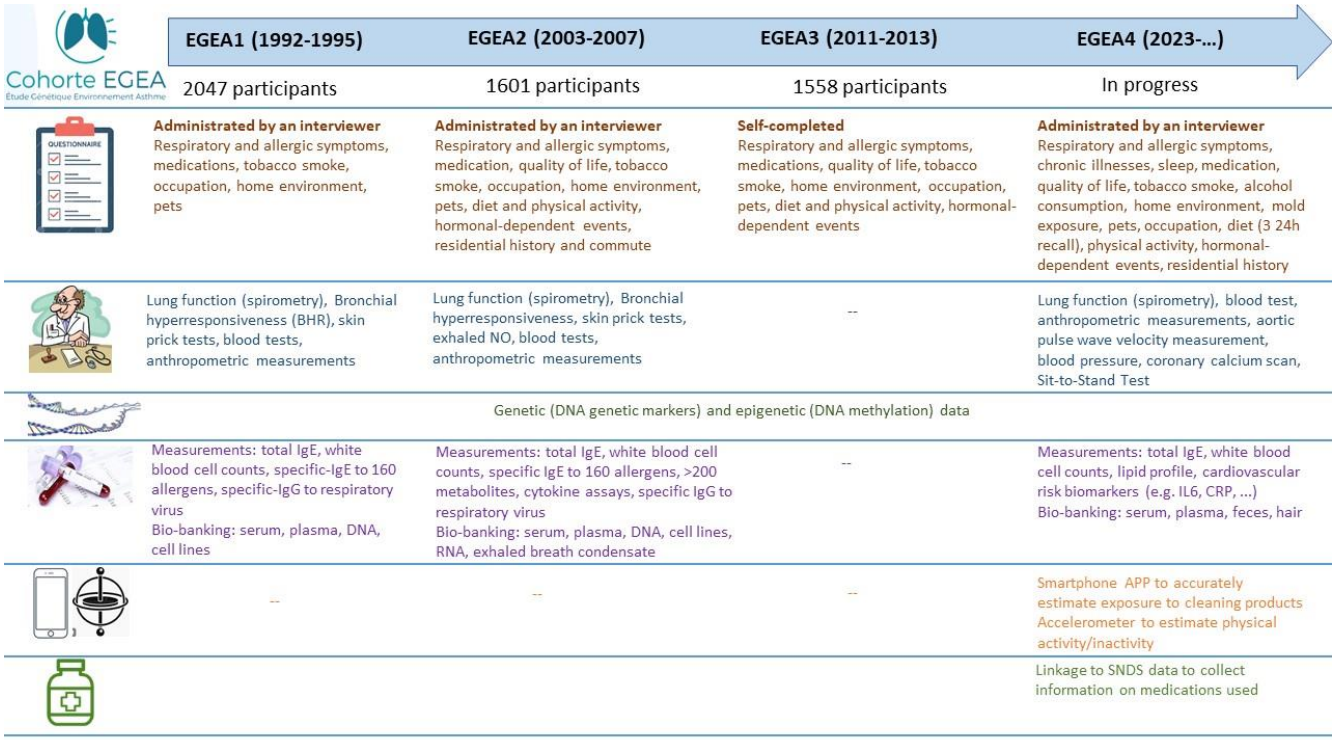

Figure 13 EGEA cohort protocol

4.2.2 Population of the study

Allergen-specific IgE (sIgE) to microarrayed allergen molecules were measured in 333 children at EGEA1 and 933 subjects EGEA2. The selection of sera was independent of total IgE, respiratory symptoms and lung function levels, limiting any risk for a selection bias. Participants with data available for sIgE, asthma status, age at asthma onset, and occupational exposure, were included in the analysis (Figure 14)

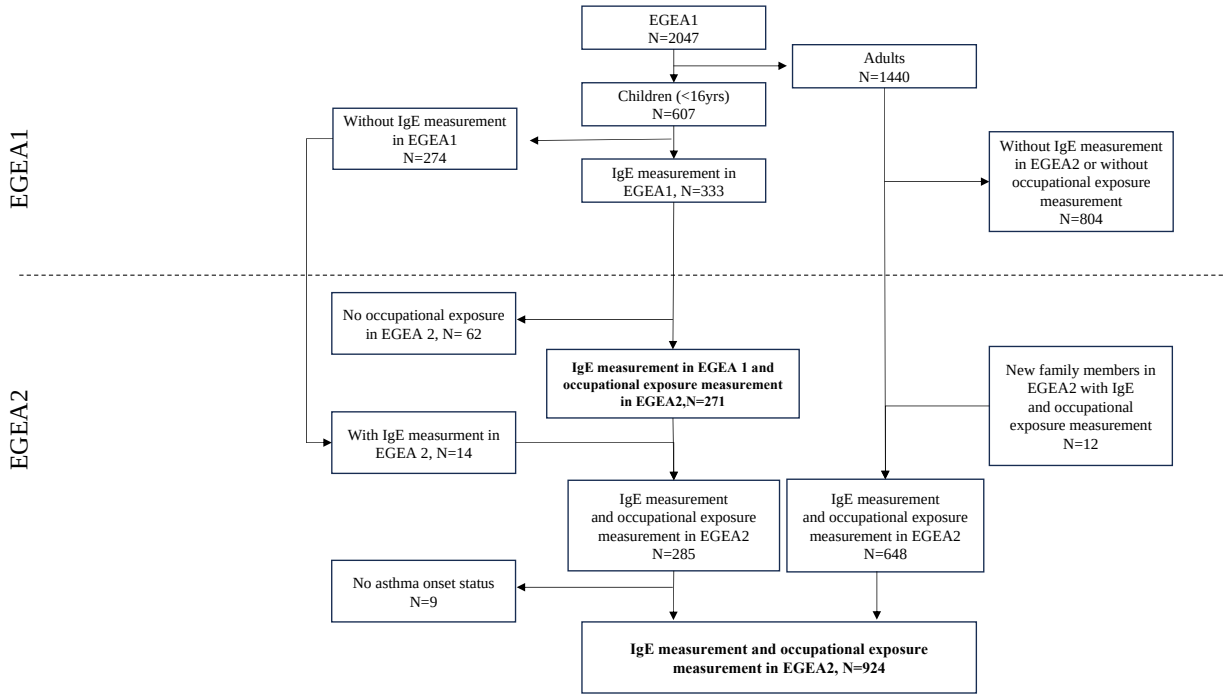

Figure 14 Flow chart of EGEA subjects included in the present analysis

4.2.3 Estimation of occupational exposures

Job-exposure matrices are tools frequently used in epidemiological studies to estimate exposure by occupation and sector of activity. They have the advantage of estimating exposure in a non-differential manner between sick and healthy subjects [359] .

Most of the time, these job-exposure matrices are developed by experts, who list high-risk exposures and assign them to occupations and/or sectors of activity [360].

An initial jobs-exposure matrix specific to asthma was set up in the 90s [361]. However, the number of asthma-causing agents identified in the literature has tripled since then. It was recently updated (OAsJEM; https://oasjem.vjf.inserm.fr/)[360] by consensus of international experts, using a standardized process involving three independent experts for each asthma-causing nuisance.

EGEA participants completed a questionnaire about their work history which was coded by an expert according to the International Standard Classification of Occupation (ISCO)-88.

We applied the occupational asthma-specific job-exposure matrix (OAsJEM) [360] which estimates exposure to 30 occupational agents divided into 3 groups: HMW sensitizers, LMW sensitizers and irritants. List of individual agents of OasJEM are illustrated table 7. Each exposure is evaluated in 3 levels: high (high probability of exposure and moderate to high intensity), medium (low to moderate probability or low intensity of exposure), no (unlikely to be exposed; low probability and low intensity).

Table 7 List of individual agents of OAsJEM classified in 3 large groups in the occupational asthma-specific JEM Agents

Agents, n=30	HMW sensitizer	LMW Sensitizer	Irritant
Animals	1		
Fish/shellfish	1		
Flour	1		
Foods	1		
Plant-related dusts	1		
House dust mites	1		
Storage mites	1		
Plant mites	1		
Enzymes	1		
Latex	1		
Textiles	1		1
Moulds			1
Endotoxin			1
Drugs	1	1	
High-level chemical disinfectants		1	1
Aliphatic amines		1	1
Isocyanates		1	1
Acrylates		1	1
Epoxy resins		1	1
Persulfates/henna		1	1
Wood		1	1
Metal		1	1
Metal working fluids		1	1
Herbicides			1
Insecticides			1
Fungicides			1
Indoor cleaning			1
Bleach			1
Organic solvents			1
Exhaust fumes			1

4.2.4 Measurements of sIgE sensitization

The field of molecular allergology has undergone considerable advancement, accompanied by parallel progress in biotechnology. These developments have collectively facilitated the creation of microarray techniques capable of detecting IgE reactivity for an extensive range of

specific allergenic compounds (>100) [362,363]. On an individual level, these techniques allow us to understand the cross-sensitisation profiles between different allergenic extracts (e.g. pollen-fruit syndrome mediated by sensitization to the PR10 protein). These microarray techniques have been used in a large European programme bringing together birth cohorts to study the mechanisms of allergy development (MedALL Mechanisms for the Development of ALLergies) [363,364].

In EGEA IgE reactivity to microarrayed allergen molecules was quantitatively determined in anonymized samples with the MeDALL-chip by a blinded operator [363].

The MeDALL-chip comprises 162 allergen components including aero- and food allergen components. sIgE data were discretized using a binary threshold (positive >0.30 ISU) [365]. To assess the number of positive sIgE response, was defined 63 clinically relevant respiratory allergenic molecules illustrated table 8 [366].

Table 8 Frequencies of sIgE recognition for the 63 allergen components studied

Species		Allergen	Molecular Group	Prevalence in EGEA 1 n=271	Prevalence in EGEA 2 N=924
Pollen	Birch	**Bet v 1**	PR-10	30 (11%)	94 (10%)
		Bet v 2	Profilin	7 (2.6%)	36 (3.9%)
		Bet v 4	Polcalcin	6 (2.2%)	24 (2.6%)
	Olive	**Ole e 1**	Ole e 1-related protein	46 (17%)	188 (20%)
		Ole e 7	nsLTP, type 1	3 (1.1%)	13 (1.4%)
		Ole e 9	Glucanase1	6 (2.2%)	12 (1.3%)
	Japanese cedar	Cry j 1	Pectate lyase	13 (4.8%)	82 (8.9%)
	Cypress	**Cup a 1**	Pectate lyase	23 (8.5%)	133 (14%)
	Plane tree	Pla a 1	Invertase Inhibitor	2 (0.7%)	11 (1.2%)
		Pla a 2	Polygalacturonases	15 (5.5%)	56 (6.1%)
		Pla a 3	nsLTP, type 1	4 (1.5%)	5 (0.5%)
	Timothy grass	**Phl p 1**	Grass group 1 (Beta-Expansin)	118 (44%)	317 (34%)
		Phl p 2	Grass group 2/3	36 (13%)	154 (17%)
		Phl p 5b	Grass group 5	49 (18%)	165 (18%)
		Phl p 6	Grass group 5/6	27 (10.0%)	121 (13%)
		Phl p 7	Calcium-binding proteins (Polcalcin)	7 (2.6%)	30 (3.2%)
		Phl p 11	Ole e 1-related protein	14 (5.2%)	66 (7.1%)
		Phl p 12	Profilin	6 (2.2%)	27 (2.9%)
	Ragweed	**Amb a 1**	Pectate lyase	13 (4.8%)	39 (4.2%)
	Mugwort	**Art v 1**	Defensin-like protein	15 (5.5%)	58 (6.3%)
		Art v 3	nsLTP, type 1	5 (1.8%)	11 (1.2%)
	Goosefoot	**Che a 1**	Ole e 1-related protein	9 (3.3%)	18 (1.9%)
	Plantain	**Pla l 1**	Ole e 1-related protein	15 (5.5%)	49 (5.3%)
	Wall pellitory	Par j 2	LTP, type 2	2 (0.7%)	16 (1.7%)
	Saltwort	Sal k 1	Pectin methylesterase	2 (0.7%)	4 (0.4%)
Latex	Latex	Hev b 1	Rubber elongation factor	30 (11%)	18 (1.9%)
		Hev b 3	Small rubber particle protein	45 (17%)	44 (4.8%)
		Hev b 5	Acidic protein	7 (2.6%)	9 (1.0%)
		Hev b 6.01	Hevein (Prohevein)	16 (5.9%)	80 (8.7%)
Molds	Alternaria	**Alt a 1**	Acidic glycoprotein	40 (15%)	83 (9.0%)
		Alt a 6	Enolase	21 (7.7%)	18 (1.9%)
	Aspergillus	Asp f 1	Mitogillin family (Ribonuclease)	2 (0.7%)	13 (1.4%)
		Asp f 3	Peroxysomal protein	1 (0.4%)	29 (3.1%)
		Asp f 6	Mn superoxide dismutase	0 (0%)	6 (0.6%)
	Cladosporium	Cla h 8	Mannitol dehydrogenase	4 (1.5%)	4 (0.4%)

	Species	Allergen	Molecular Group	Prevalence in EGEA 1 n (%)	Prevalence in EGEA 2 n (%)
Mites	**House dust mite**	**Der p 1**	Group 1 mite allergens (Cysteine protease)	99 (37%)	237 (26%)
		Der p 2	Group 2 mite allergens (NPC2 family)	115 (42%)	273 (30%)
		Der p 4	Group 4 mite allergens (Alpha-amylase)	74 (27%)	117 (13%)
		Der p 7	Group 7 mite allergens	73 (27%)	147 (16%)
		Der p 10	Group 10 mite allergens (Tropomyosin)	89 (33%)	121 (13%)
		Der p 11	Group 11 mite allergens (Paramyosin)	35 (13%)	39 (4.2%)
		Der p 14	Vitellogenin (Apolipophorins)	24 (8.9%)	14 (1.5%)
		Der p 15	Chitin-binding domain	20 (7.4%)	22 (2.4%)
		Der p 18	Chitin-binding domain	25 (9.2%)	40 (4.3%)
		Der p 21	Group 5/21 mite allergens	54 (20%)	103 (11%)
		Der p 23	Chitin-binding domain	113 (42%)	239 (26%)
		clone 16	Chitin-binding domain	28 (10%)	66 (7.1%)
	Storage mite	**Lep d 2**	Group 2 mite allergens (NPC2 family)	17 (6.3%)	33 (3.6%)
	Blomia tropicalis	**Blo t 5**	Group 5/21 mite allergens	25 (9.2%)	46 (5.0%)
	Cockroach	Bla g 1	Cockroach group 1	0 (0%)	4 (0.4%)
		Bla g 2	Aspartic protease	1 (0.4%)	1 (0.1%)
		Bla g 5	Glutathione S-transferase	0 (0%)	0 (0%)
	Anisakis	Ani s 1	Serine protease inhibitor	2 (0.7%)	9 (1.0%)
Animals	**Cat**	**Fel d 1**	Uteroglobin	65 (24%)	228 (25%)
		Fel d 2	Serum Albumin	6 (2.2%)	39 (4.2%)
		Fel d 4	Lipocalin	17 (6.3%)	49 (5.3%)
	Dog	**Can f 1**	Lipocalin	17 (6.3%)	65 (7.0%)
		Can f 2	Lipocalin	7 (2.6%)	23 (2.5%)
		Can f 4	Lipocalin (Odorant-binding protein)	9 (3.3%)	30 (3.2%)
		Can f 5	Arginine Esterase (Trypsin-like serine protease)	1 (0.4%)	19 (2.1%)
		Can f 6	Lipocalin	7 (2.6%)	30 (3.2%)
	Horse	**Equ c 1**	Lipocalin	14 (5.2%)	42 (4.5%)
	Mouse	**Mus m 1**	Lipocalin	20 (7.4%)	36 (3.9%)

Results expressed in n (%); Allergens in bold represent allergens used for latent component analysis

4.2.5 Strategy of analysis and statistical tool

The study aimed to assess the association between IOE (Irritant Occupational Exposure) and sIgE sensitization according to asthma status (childhood onset asthmatic, adult-onset asthmatic, and non-asthmatic participants). In order to do so, cross-sectional association between IOE and sIgE sensitization was evaluated among adult participants in EGEA2.

Measuring the association between OEI and IgE sensitization could be challenging because of a possible healthy hire effect; sensitized participants during childhood could avoid jobs associated with OIE [367,368]. That's why the longitudinal association between childhood sIgE sensitization and IOE during adulthood was assessed in EGEA1 participants with follow up data at EGEA2.

Concerning IOE, was studied lifetime occupational exposures to irritant. Each exposure was assessed in two categories (ever exposed vs. never exposed) or three categories (medium exposure, high exposure, never exposed).

sIgE sensitization was expressed in both profile of sIgE sensitization and number of positive sIgE responses. To assess the number of positive sIgE response, was defined 63 clinically relevant respiratory allergenic molecules that are illustrated table 8. To identify sIgE sensitization profiles, Latent Class Analysis (LCA), a data-driven approach, was applied on IgE-reactivity as in previous work [68]. However, the number of allergens used and the number of clusters were different in order to avoid missing data and to have enough individuals in each group to allow multivariate analysis. Indeed, LCA was applied to 37 *a priori* most relevant respiratory allergens, being recognized by enough participants (n>10), without bringing redundant information, without missing data. LCA was applied on IgE reactivity of these 37 allergens. Each participant was assigned to the latent cluster (here defined as sIgE sensitization profile) for which he/she had the highest membership probability. The criteria for selecting the

number of clusters were: 1) the model with the lowest Bayesian information criterion [369] 2) the model with number of observation for each cluster greater than 10% of the total number of observations.

The cross-sectional association between the level of occupational irritant exposure at EGEA 2 and the number of sIgE sensitization at EGEA2 according to asthma status was assessed by negative binomial analysis. This regression models the ratio ("adjusted mean ration" aMR) of the mean number of positive sIgE sensitization between participants exposed and not exposed to OIE, independently to adjustments variables. For example, an aMR of 0.5 for OIE means that participants exposed to irritants exhibit a mean number of positive sIgE twice as low than those who are not exposed. The cross-sectional association of occupation irritant exposure at EGEA 2 with the type of sensitization at EGEA2 according to asthma status was assessed by multinomial logistic regression. Adjustment was performed for age, sex, smoking habit and early country living. A sensitivity analysis was performed after exclusion of participants co-exposed to HMW agents.

Longitudinal analysis of the association of sIgE sensitization at EGEA 1 with OIE at EGEA 2 was assessed by multinomial logistic regression and logistic regression with adjustment for age and sex and father diploma level. The missing data rate was respectively 0.9 and 3.5% for the cross sectional and the longitudinal analysis. Multiple imputation by chained equation was undergone for missing data using mice package in R.

General Results

5 Occupational Asthma Caused by Quaternary Ammonium Compounds: A Multicenter Cohort Study.

Nicolas Migueres, MD[a,b], Olivier Vandenplas, MD PhD[c], Charlotte Debaille, MD[c], Jolanta Walusiak-Skorupa, MD[d], Marta Wiszniewska, MD[d], Xavier Munoz, MD[e], Vera van Kampen, PhD[f], Hille Suojalehto, MD, PhD[g], Katri Suuronen, PhD[g], Martin Seed, MD[h], Sewon Lee, MPH[h], Catherine Rifflart, MSc[c], Julien Godet, PharmD, PhD[a], Frédéric de Blay, MD[b], on behalf of the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) *

Authors' affiliation:

[a] Groupe Méthode Recherche Clinique, Pôle de Santé Publique, Strasbourg University, Strasbourg, France;

[b] Division of Asthma and Allergy, Department of Chest Diseases, University Hospital of Strasbourg and Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France;

[c] Department of Chest Medicine, Centre Hospitalier Universitaire UCL Namur, Université Catholique de Louvain, Yvoir, Belgium;

[d] Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Lodz, Poland;

[e] Servei Pneumologia, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona and CIBER de Enfermedades Respiratorias (CIBERES), Barcelona, Spain;

[f] Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Ruhr University, Bochum, Germany;

[g] Occcupational Health, Finnish Institute of Occupational Health, Helsinki, Finland

[h] Centre for Occupational and Environmental Health, The University of Manchester, Manchester, United Kingdom;

Contribution: This work was published in the *The Journal of Allergy and Clinical Immunology: In Practice*. I was involved in the collection of data in Strasbourg, I performed the statistical analyses, and wrote the first draft. Data managements was performed by Namur investigator (OV, CR). The identification of QAC patients by the QSAR model utilization was performed by Namur investigator

5.1 Abstract

Background: Quaternary ammonium compounds (QACs) are used extensively for cleaning and disinfection and have been documented in scatttered reports as a cause of occupational asthma (OA) through SIC.

Objective: To examine the clinical, functional, and inflammatory profile of QAC-induced OA compared to OA caused by other low-molecular-weight (LMW) agents.

Methods: The study was conducted in a retrospective multicenter cohort of 871 subjects with OA ascertained by a positive SIC. Subjects with QAC-induced OA (n=22) were identified based on a positive SIC to QACs, after exclusion of those challenged with cleaning products or disinfectants that contained other potential respiratory sensitizers, and they were compared to 289 subjects with OA caused by other LMW agents.

Results: Most subjects with QAC-induced OA were working in the healthcare sector (n=14). A ≥2-fold increase in the postchallenge level of nonspecific bronchial hyperresponsiveness was recorded in 8 of 11 (72.7%) subjects with QAC-induced OA, and in 49.7% of those with OA due to other LMW agents. Although sputum assessment was available in only 8 subjects with QAC-induced OA, they showed a significantly greater median (interquartile) increase in sputum eosinophils (18.1% [12.1 to 21.1]) compared to those with OA due to other LMW agents (2.0% [0 to 5.2], $P<0.001$).

Conclusion: This study indicates that QAC-induced OA is associated with a highly eosinophilic pattern of airway response and provides further evidence supporting the sensitizing potential of QACs. The findings highlight the heterogeneous nature of the pathobiological pathways involved in OA caused by LMW agents.

5.2 French Summary

Contexte : Les ammoniums quaternaires (AQ) sont largement utilisés pour le nettoyage et la désinfection et ont été documentés ponctuellement comme une cause d'asthme professionnel (AP) par le biais de tests de provocation bronchique (TPB)

Objectif : Examiner le profil clinique, fonctionnel et inflammatoire de l'asthme induite par les AQ par rapport à l'asthme induit par d'autres agents de bas poids moléculaire (BPM).

Méthodes : L'étude a été menée dans une cohorte multicentrique rétrospective de 871 sujets souffrant d'asthme et dont le diagnostic a été établi à l'aide d'un TPB positif. Les sujets atteints d'AP induit par les AQ (n=22) ont été identifiés sur la base d'un TPB positif aux AQ, après exclusion des sujets exposés à des produits de nettoyage ou à des désinfectants contenant d'autres sensibilisants respiratoires potentiels, et ils ont été comparés à 289 sujets atteints d'AP causé par d'autres agents de BPM.

Résultats : La plupart des sujets souffrant d'AP induit par des AQ travaillaient dans le secteur de la santé (n=14). Une augmentation ≥2 fois du niveau d'hyperréactivité bronchique non spécifique après le TPB a été enregistrée chez 8 des 11 (72,7 %) sujets atteints d'asthme induit par le QAC, et chez 49,7 % de ceux atteints d'asthme induit à d'autres agents de BPM. Bien que l'évaluation des expectorations n'ait été disponible que chez 8 sujets atteints d'asthme induite par les AQ, l'augmentation médiane (interquartile) des éosinophiles dans les expectorations induites était significativement plus importante (18,1 % [12,1 à 21,1]) par rapport à ceux atteints d'asthme induit à d'autres agents de BPM (2,0 % [0 à 5,2], P<0,001).

Conclusion : Cette étude indique que l'asthme induit par les AQ est associé à un type d'inflammation bronchique fortement éosinophilique et fournit des preuves supplémentaires

du potentiel sensibilisant des AQ. Les résultats soulignent la nature hétérogène des mécanismes physiopathologique de l'asthme professionnel provoquée par des agents de BPM.

5.3 Introduction

Quaternary ammonium compounds (QACs) have the generic chemical structure $N(R_1R_2R_3R_4)^+ Cl^-$, where R_{1-4} are alkyl or aryl groups with varying carbon chain length. These compounds are used extensively for the cleaning and disinfection of surfaces, instruments, and equipment, especially in healthcare and food processing facilities, because of their broad-spectrum antimicrobial activity [370,371] . Asthmatic reactions induced by the most widely used QACs, benzalkonium chloride (Chemical Abstracts Service [CAS] #8001-54-5) and didecyl dimethyl ammonium chloride (CAS #7173-51-5), have been documented through SIC in a few case reports[372–374] and limited case series [375,376]. A physician-based notification scheme of work-related asthma in France reported a significant upward trend in incident cases attributed to QACs from 1.4% of reported cases in 2001 to 8.3% in 2009, mainly in the health and social sectors [377]. Nevertheless, epidemiological surveys of cleaners either did not to address specifically exposure to QACs [378–382] or failed to document an association between asthma and exposure to QACs [383,384], with the notable exception of Gonzalez et al. [385] who found an association between exposure to QACs and increased risk of asthma in a survey of healthcare workers. Overall, the role of QAC exposure in the development of asthma remains largely unknown and controversial[386,387].

This study aimed to characterize the clinical, functional, and inflammatory profiles of occupational asthma (OA) caused by QAC ascertained by a positive SIC and to compare these phenotypic patterns with those of OA due to other low-molecular-weight (LMW) agents.

5.4 Methods

5.4.1 Study design and population

This retrospective, observational study was conducted in a cohort of 871 subjects who showed a positive SIC with various occupational agents between January 2006 and December 2018 in six centers participating in the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS)[280,294,299,388] . This analysis was restricted to six E-PHOCAS centers which were selected based on the performance of induced sputum analysis before and after SIC, although this technique was not available throughout the whole 2006-2018 study period in each center. The recruitment of the population included in this analysis on cleaners' OA is described in Figure 15 and Table 9 in the supplements.

The data collection process used by the E-PHOCAS cohort has previously been described [280,294,299,388]. Briefly, detailed anonymized information on demographic, clinical, occupational, and physiological characteristics of the subjects at the time of the diagnostic evaluation was entered in a standardized Excel spreadsheet in each participating center. The requested data were retrieved from medical charts in 2 centers while in the other centers, all or most of the data had been prospectively entered in existing local databases. At the time of data collection, the local investigators were not aware of the specific aims of the analyzes that would be conducted subsequently. Important outcomes, such as the results of SIC, asthma severity, and level of nonspecific bronchial hyperresponsiveness (NSBH) were interpreted and recoded a posteriori using uniform and validated criteria. The local databases were checked for missing data and inconsistencies by 3 investigators (O.V., C.R., and J.D.), pooled together, and centralized at the Strasbourg University.

5.4.2 Ethics

Approval for this retrospective analysis of anonymized data was obtained from each local Institutional Review Board. The central database at the Strasbourg University was approved by the "*Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé*" and the "*Commission Nationale de l'Informatique et des Libertés*".

5.4.3 Identification of asthma induced by QACs

First, the subjects with occupational asthma (OA) caused by cleaning agents were retrieved by screening the recorded occupation and causal agent fields of the E-PHOCAS database. A cleaning agent was defined as any material used for cleaning or disinfecting houses, buildings, healthcare equipment, or specialised service and industrial facilities, with the exceptions of degreasing metal parts [389] and textile dry cleaning. In case of doubt, further information on job tasks and products used at work were requested from the local investigators. This resulted in the identification of 55 subjects with cleaners's OA.

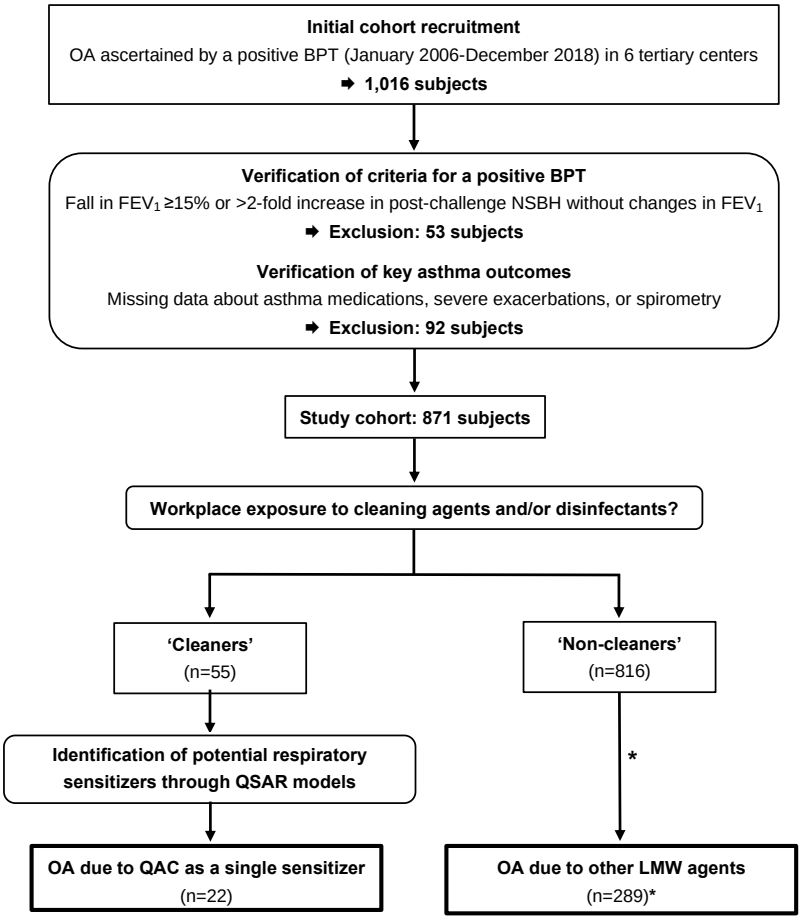

Figure 15 Flowchart of the study

bronchoprovocation tests; *FEV1*, forced expiratory volume in one-second; *LMW*, low molecular weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OA*, occupational asthma; *QAC*, quaternary ammonium compound; QSAR, quantitative structure-activity relationship

* Twenty-six subjects with acrylate-induced OA were excluded from the initial cohort since this subset has been recently documented as demonstrating a distinct phenotype compared to other LMW agents[294]. Nine subjects with OA due to aldehydes used for non-cleaning purposes were also excluded**.**

OA caused by QACs was defined by a positive SIC response induced by a QAC alone, that is in the absence of any other potential respiratory sensitizer. In order to identify these subjects with QAC-induced OA, the safety data sheets of the cleaning products that elicited positive SIC were reviewed. The respiratory sensitization potential of their ingredients was assessed using a validated quantitative structure-activity relationship (QSAR) model [389,390] that generates quantitative estimates of the probability that LMW organic agents have respiratory sensitization potential based on their chemical structure (i.e., the "asthma hazard index") (see supplementary at the end }. Based on this approach, the subjects were assigned to one of three categories: 1) positive SIC induced by a single respiratory sensitizing agent (n=30); 2) positive SIC elicited by challenge exposure to one or more products containing multiple potential sensitisers (n=21); and 3) positive SIC without identified sensitizer (n=4). The potential respiratory sensitizers involved in the 55 subjects with cleaner's asthma are detailed in Table 4. The single-sensitizer positive SIC were induced by QACs in 22 subjects, including didecyldimethylammonium chloride (n=16) and benzalkonium chloride (n=6). Of note, no respiratory sensitizer was identified in four subjects with a positive SIC. The characteristics of these four subjects are summarized in Table 11 in supplements. The 22 subjects with QAC-induced OA were compared to 289 subjects with OA caused by various other LMW agents (Table 5).

5.3.4 Demographic and clinical characteristics

The E-PHOCAS used a standardized spreadsheet to gather information on the following: 1) causal agents and job; 2) demographic and clinical characteristics; 3) timing of work-related respiratory symptoms in relation to exposure to the causal agent; 4) co-existing conditions (i.e. physician-based diagnosis of work-related rhinitis, contact urticaria and/or dermatitis, and sinusitis); and 5) materials and methods used for SIC performance.

5.3.5 Lung function assessments

The database collected the baseline forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) values as well as the level of nonspecific bronchial hyperresponsiveness (NSBH) measured at baseline and 24 hours after challenge exposure. The level of NSBH was categorized as “absent”, “mild”, or “moderate/severe” according to the bronchoprovocation method used in each center [280,299] (see general methods). A significant increase in post-challenge NSBH was defined by a ≥2-fold increase in the level of NSBH measured 24 hours after the challenge exposure as compared to the baseline value (i.e., a baseline/post-challenge ratio of NSBH indices ≥2) [284].

5.3.6 Specific inhalation challenge with occupational agents

SIC conformed with international recommendations in terms of safety precautions, “placebo” challenge, and duration of functional monitoring (see general Methods) [284]. A positive SIC result was defined by either a ≥15% fall in FEV1 at any time during the post-challenge monitoring period or a ≥2-fold increase in the post-challenge level of NSBH as compared to baseline value [284].

The SIC with QACs aimed to recreate as close as possible the conditions of exposure at the workplace by wiping/brushing (n=21) and/or spraying (n=14) the commercial product that contained QAC as the single potential sensitizer (n=13) or a pure QAC solution (n=9). The products were diluted in water as recommended by the manufacturer.

Table 1 :Potential respiratory sensitizers involved in positive bronchoprovocation tests with cleaning and disinfecting products

Single sensitizer (n=30):	
Quaternary ammonium compounds	
Didecyl dimethyl ammonium chloride (CAS# 7173-51-5)	16
Benzalkonium chloride (CAS# 8001-54-5)	6
Amines	
Ethanolamine (CAS# 141-43-5)	2
Ethylenediamine (CAS# 107-15-3)	2
Glutaraldehyde (CAS# 111-30-8)	3
Chloramine T (CAS# 127-65-1)	1
Multiple potential sensitizers (n=21):	
Quaternary ammonium compounds*	
Didecyl dimethyl ammonium chloride (CAS# 7173-51-5)	12
Benzalkonium chloride (CAS# 8001-54-5)	10
Amines	
Ethylenediaminetetraacetic acid (CAS# 60-00-4)	4
Lauryldimethylamine oxide (CAS# 1643-20-5)	3
N-(3-aminopropyl)-N-dodécylpropane-1,3-diamine) (CAS# 2372-82-9)	1
Tetraacetylethylenediamine (CAS# 10543-57-4)	1
C12-14-alkyltrimethylenediamine (CAS# 90640-43-0)	2
N,N-dimethyltetradecylamine N-oxide (CAS# 3332-27-2)	1
Glutaraldehyde (CAS# 111-30-8)	9
Chloramine T (CAS# 127-65-1)	7
Chlorhexidine (CAS# 55-56-1)	6
Polyhexanide (CAS# 28757-47-3)	5
Octenidine dihydrochloride (CAS# 70775-75-6)	2
1,2-Benzisothiazol-3(2H)-one (CAS#2634-33-5)	2
Sodium dichloroisocyanurate dehydrate (CAS# 51580-86-0)	2
Sodium dodecylbenzene sulphonate (CAS# 25155-30-0)	3
Enzymes (not detailed)	3
No identified sensitizer (n=4)	

Legend: Potential sensitizers identified using a quantitative structure-activity relationship (QSAR) model[389] . *CAS*, Chemical Abstracts Service.

***** Among 21 positive bronchoprovocation tests performed with multiple potential sensitizers, 20 were challenged with products containing quaternary ammonium compounds while one subject showed a positive reaction to a cleaning product containing chloramine T and N,N-dimethyltetradecylamine.

5.3.7 Markers of airway inflammation

Data pertaining to markers of airway inflammation included, whenever available: 1) baseline blood eosinophils (assessed within one month of the SIC procedure); 2) sputum eosinophils

and neutrophils expressed as a percentage of total cell count at baseline and 24 hours post-challenge; and 3) fractional exhaled nitric oxide concentration (FeNO) at baseline and 24 hours after the SIC. Detailed information on the methodology used for sputum induction and processing in the participating centers is available in general methods. An "eosinophilic response" was defined as a postchallenge increase in sputum eosinophil count ≥2% (post-SIC minus baseline percentage value). The FeNO level was measured in five of the six centers according to recommendations from both the European Respiratory Society and the American Thoracic Society recommendations [391].

Table 2:Low-molecular-weight agents involved in occupational asthma in the study cohort

LMW agents	**n**
Isocyanates	97
Persulfate salts	53
Metals	26
Wood dusts	25
Welding	22
Metal working fluids	10
Amines	10
Acid anhydrides	9
Epoxy resins	7
Drugs	6
Resins/glues/paints (NOS)	5
Colophony	5
Reactive dyes	2
Styrene	1
Various LMW agents	11
Total:	289

Legend: *LMW*, low-molecular-weight; *NOS*, not otherwise specified.

5.4 Data analysis

Quantitative data are presented as a median and interquartile range (IQR). Comparison between groups of subjects was made using the Fisher's exact or chi-squared test for categorical variables and non-parametric tests for numerical variables. A multivariable linear regression analysis was conducted using a generalized linear model and a stepwise procedure based on the Akaike information criterion to select the most parsimonious model among subjects with available sputum assessment and a positive SIC to QACs or other LMW agents (n=79) in order to explore the factors that determined the magnitude of the post-SIC change in sputum eosinophils (i.e. the difference between post- and pre-SIC sputum eosinophil count expressed in % of total nonsquamous cells). The potential confounding factors (i.e. independent variables) incorporated into this regression included: a positive SIC induced by QACs (yes/no); age; gender; smoking status (current and ex-smokers vs. never smokers); treatment with inhaled corticosteroid at the time of the SIC (yes/no); baseline sputum eosinophil count (% total nonsquamous cells); and the time elapsed since last work exposure (≤1 mo vs. > 1 mo). Statistical analysis was performed using the R software version 3.4.1 (*https://cran.r-project.org*). A *P*-value <0.05 was considered significant.

5.5 Results

5.5.1 Clinical and occupational characteristics

Twenty-two subjects with a positive SIC to QACs as the single identified sentizer, including benzalkonium chloride (n=6) and didecyl dimethyl ammonium chloride (n=16) were identified. These subjects were most commonly working in healthcare environments (n=14), including cleaners (n=6), nurses (n=5), one hospital technologist, one dental assistant and one administrative worker, or in food processing facilities (n=4). Another two subjects were

domestic cleaners and two subjects were employed in educational services. Notably, two of these subjects were administrative employees with indirect exposure to cleaning products in a healthcare facility and a school.

The clinical and functional characteristics of the subjects with OA caused by QACs are summarized in Table 6. In comparison with OA due to other LMW agents, QAC-induced OA was associated with a significantly higher proportion of women and never-smokers. Subjects with QAC-induced OA showed greater median FVC and FEV1 values while the FEV1/FVC ratio did not differ between the groups. There was no difference in the level of baseline NSBH or the pattern of asthmatic reactions during the SIC between subjects with OA due to QACs and those challenged with other LMW agents. Among subjects with QAC-induced OA, a ≥2-fold increase in the post-challenge level of NSBH was recorded in eight of 11 (72.7%) subjects with an available post-challenge measurement of NSBH, a proportion that tended to be higher but not significantly from OA due to other LMW agents (88 of 177, 49.7%).

5.5.2 Markers of airway inflammation

Suitable pre- and post-challenge sputum samples were available in only 8 of 22 (36.4%) subjects with QAC-induced OA and in 71 of 289 (24.6%, P=0.220) subjects with a positive SIC to other LMW agents (see Table 10). The median (IQR) baseline sputum eosinophil count was slightly higher (6.0% [2.0-10.0]) in the 8 subjects with QAC-induced OA than in those with OA due to other LMW agents (1.0% [0.6-5.0], P=0.052) (Table IV). Positive SICs with QACs were associated with a significantly greater median (IQR) post-challenge increase in sputum eosinophils (18.1% [12.1 to 21.1]) compared to the other LMW agents (2.0% [0-5.2], P<0.001). An “eosinophilic response” (i.e. ≥2% postchallenge increase in sputum eosinophils) was significantly more frequent (8 of 8 subjects) in QAC-induced OA than in

subjects with a positive SIC elicited by other LMW agents (37 of 71, 52.1%, *P*=0.009). The multivariate regression analysis retained only a positive SIC induced by QACs as a factor associated with a greater increase in post-SIC sputum eosinophils (Table 8). There were no differences between OA caused by QACs and the other LMW agents with regard to baseline blood eosinophil counts as well as the baseline and post-challenge FeNO values.

Table 3 : Clinical and functional characteristics of subjects with occupational asthma caused by quaternary ammonium compounds compared to other low-molecular-weight agents

Characteristic	**OA due to QACs (n=22)**	**OA due to other LMW agents (n=289)**	***P*-value**
Age (yr)*	45 (40-52)	44 (34-52)	0.295
Sex (female)	19 (86.4)	115 (39.8)	**<0.001**
Body mass index (kg/m^2)	28.6 (22.8-33.03)	26.7 (24-29.8)	0.536
Smoking habits:			**0.016**
Current/ex-smokers	6 (27.2)	157 (54.3)	
Never-smoker	16 (72.7)	132 (45.7)	
Atopy†	11 (50.0)	117 (42.1)	0.507
Asthma pre-existing to the causal exposure	3 (13.6)	28 (9.7)	0.470
Duration of exposure before asthma onset (mo)*	75 (12-150)	82 (30-193)	0.245
Duration of symptomatic exposure (mo)*	28 (8-54)	29 (12-38)	0.621
Interval since last work exposure (mo)*	0.6 (0.1-6)	1.7 (0.1-10.0)	0.775
Coexisting conditions:			
Work-related rhinitis	10 (45.5)	167 (58.0)	0.271
Chronic rhinosinusitis	4 (18.2)	37 (12.8)	0.510
Work-related urticaria	2 (9.1)	24 (8.4)	0.706
Work-related contact dermatitis	5 (22.7)	37 (12.9)	0.198
Asthma treatment/severity at work‡			
No treatment	3 (13.6)	48 (16.6)	0.283
Mild (GINA treatment step 1-2)	7 (31.8)	44 (15.2)	
Moderate(GINA treatment step 3)	6 (27.3)	87 (30.1)	
Severe (GINA treatment step 4-5)	6 (27.3)	110 (38.1)	
Inhaled short-acting β_2-agonist ≥1/day	6 (27.2)	97 (33.6)	0.643
≥1 severe asthma exacerbation (last 12 mo)	7 (31.8)	79 (27.3)	0.628
Baseline spirometry			
FVC, % pred*	108 (100-114)	96 (86-107)	**0.002**
FEV1, % pred*	96 (89-110)	89 (80-98)	**0.029**
FEV1 <80%	4 (18.2)	72 (24.9)	0.611
FEV1/FVC*	78 (73-82)	76 (70-81)	0.390
FEV1/FVC <70%	4 (18.2)	70 (24.2)	0.614
Maximum fall in FEV1 (% from baseline value)	22 (18-27) §	23 (18-29) §	0.441
Baseline level of NSBH	(n=22)	(n=272)	0.916
Absent	5 (22.7)	76 (27.9)	
Mild	12 (54.5)	138 (50.7)	
Moderate-to-severe	5 (22.7)	58 (21.3)	
Post-challenge change in NSBH	(n=11)	(n=177)	
Pre/post-challenge NSBH ratio*	3.0 (1.5-4.1)	2.0 (1.0-4.0)	0.289
Pre/post-challenge NSBH ratio ≥2	8 (72.7)	88 (49.7)	0.214
Pattern of bronchial response to SIC	(n=20) §	(n=255) §	0.729
Isolated early reaction	7 (31.8)	61 (21.7)	
Isolated late reaction	7 (31.8)	99 (35.2)	
Dual reaction	6 (27.3)	95 (33.8)	

Legend: *SIC*, bronchoprovocation tests; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *LMW*, low molecular weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OA*, occupational asthma; *QAC*, quaternary ammonium compound. *GINA*, Global Initiative for Asthma. Data are presented as n (% of available data) unless otherwise specified. Values in boldface are statistically significant.

* Median value with interquartile range (IQR) within parentheses.
† Atopy defined by the presence of at least one positive skin prick test result to common allergens.
‡ The severity of asthma was graded according to the treatment steps proposed by the Global Initiative for Asthma [351] as “untreated” (step 0); “mild” (step 1-2); “moderate” (step 3); and “severe” (step 4-5).

Table 4:Airway inflammation markers in subjects with OA caused by quaternary ammonium compounds compared to other low-molecular-weight agents

Characteristic	OA due to QACs (n=22)	OA due to other LMW agents (n=289)	P-value
Blood eosinophils	(n=13)	(n=150)	
Cells/µl*	244 (190 to 460)	229 (159 to 400)	0.451
>300/µl	6 (46.2)	56 (37.3)	0.561
Baseline FeNO	(n=15)	(n=98)	
ppb*	23 (13 to 38)	19 (10 to 31)	0.528
Postchallenge FeNO			
ppb*	25 (15 to 50)	26 (15 to 52)	0.912
Change (ppb)*	4 (-1 to 7)	6 (1 to 18)	0.324
Baseline sputum eosinophils	(n=8)	(n=71)	
%*	6.0 (2.0 to 10.0)	1.0 (0.6 to 5.0)	**0.052**
≥3%	5 (62.5)	26 (36.6)	0.252
Postchallenge sputum eosinophils			
%*	24.2 (17.6 to 29.2)	4.0 (2.0 to 9.0)	**<0.001**
Change compared to baseline value (%)*	18.1 (12.1 to 21.1)	2.0 (0 to 5.2)	**<0.001**
Increase ≥2%	8 (100)	37 (52.9)	**0.009**
Baseline sputum neutrophils	(n=8)	(n=71)	
%*	46.5 (36.4 to 60.1)	55 (40 to 68)	0.597
Post-challenge sputum neutrophils			
%*	51.8 (46.7 to 60.8)	56 (35 to 68)	0.715
Change compared to baseline value (%)*	3.0 (-7.4 to 6.4)	1.0 (-12.1 to 14.4)	0.726

Legend: *SIC Specific Inhalation Challenge*, *FeNO*, fractional exhaled nitric oxide; LMW, low-molecular-weight; *OA*, occupational asthma; *QAC*, quaternary ammonium compound. Data are presented as n (% of available data) unless otherwise specified. Values in boldface are statistically significant (P <0.05).

* Median value with interquartile range (IQR) within parentheses.

Table 5: Multivariate regression analysis for the changes in post-challenge sputum eosinophil count

Independent variables	**Adjusted βcoefficient (95% confidence interval)**	**P-value**
Challenge with QAC as single-sensitizer (yes/no)	10.778 (3.625 to 17.932)	**0.004**
Age (yr)	0.069 (-0.114 to 0.252)	0.457
Gender (female)	2.663 (-2.015 to 7.340)	0.260
Current and ex-smokers vs. never smokers	-3.185 (-7.367 to 0.996)	0.133
Treatment with inhaled corticosteroid at the time of SIC (yes/no)	2.146 (-2.226 to 6.517)	0.331
Baseline sputum eosinophil count (%)	--0.054 (-0.379 to 0.271)	0.742
Time elapsed since last work exposure (≤1 mo vs. >1 mo)	0.506 (-3.519 to 4.530)	0.803

Legend:*SIC Specific Inhalation Challenge*; *QAC*, quaternary ammonium compound
This multivariate linear regression model incorporated 79 subjects with available sputum analysis and a positive BPT to QAC or other low-molecular-weight agents. Bold indicates statistical significance (*P* <0.05).

5.5 Discussion

As far as we know, this study is the first attempt at characterizing the clinical, functional, and inflammatory pattern of QAC-induced OA. The findings indicated that challenge exposure to QAC is associated with an increase in NSBH and a highly eosinophilic airway response, features that are consistent with an immunologically-mediated sensitizing mechanism.

Although a number of reports have documented asthmatic reactions after challenge exposure to QACs [372–376], the inflammatory pattern induced by these chemicals has never been specifically investigated. Previous studies reported an increase in sputum eosinophils after challenge exposure to the causal agent in subjects with OA independently from the type of

agents (i.e., LMW vs. high-molecular-weight agents) and the pattern of asthmatic reaction (i.e., late vs. early reactions) [249,280]. Nevertheless, a previous analysis of the E-PHOCAS cohort demonstrated that SIC with acrylate compounds were more frequently (88%) associated with a significant increase in sputum eosinophils compared to other LMW agents (48%)[294]. On the other hand, acrylate-induced OA was more frequently associated with work-related rhinitis and acrylate compounds elicited a significantly higher increase in post-challenge FeNO compared to other LMW agents, whereas QACs failed to induce such changes in FeNO. Taken together, these observations highlight the heterogeneous nature of OA caused by LMW agents and the need to further explore differences in underlying pathobiological pathways. Interestingly, it has been recently demonstrated in a murine model that dermal exposure to the QAC dodecyl dimethyl ammonium bromide can induce the activation of type 2 innate lymphoid cells (ILC2s) in the skin [392]. Murine models of allergic asthma have shown that ILC2s are a potent source of the T_H2 cytokines IL-5 and IL-13 and are able to induce eosinophil recruitment, mucus hypersecretion, and NSBH [393], although the role of ILC2s in the development of airway sensitization to LMW chemicals warrants further investigation. Alternative mechanisms including neurogenic inflammation and mast cells degranulation resulting from direct stimulation of chemoreceptors at nerve endings, especially the transient receptor potential channels, remain purely speculative in the case of QACs[394].

The immunological mechanisms involved in the inception of OA caused by QACs remain largely unknown. There are several reports of urticaria caused by QACs that may support the possibility of an immediate-type, IgE-mediated, allergic mechanism[395]. However, in previous reports of subjects with QAC-induced OA, skin-prick tests with QACs elicited an immediate skin response in only one subject with associated urticaria, while these tests were not completed in other cases[372,374,376]. Specific IgE antibodies against “quaternary

ammonium" were either not detected [373,374] or their presence did not correlate with asthma symptoms [385]. Skin-prick tests or determination of specific IgE have not been attempted in the subjects included in our cohort. It is currently acknowledged that LMW agents causing asthma are incomplete antigens (i.e. haptens) that combine with amino acid residues on airway proteins to become immunogenic[285,396]. However, the potential diversity of chemical interactions with airway proteins could explain heterogeneous pathobiological responses and our inability to identify specific IgE in OA caused by most LMW agents.

Most subjects with QAC-induced OA (63.6%) in our series were exposed to QACs in healthcare environments. Exposure to cleaning and disinfecting products in healthcare settings has been associated with an increased risk of new-onset asthma in nurses and related occupations [379,380] with current asthma in hospital cleaners [381], and with work-related asthma symptoms in healthcare professionals [382]. These studies revealed broad categories of tasks or products associated with asthma, such as "general purpose cleaning" and "instrument cleaning/sterilisation" [380,382,397] but they failed to identify specific agents involved in asthma onset. Using a specific job-task-exposure matrix, the Nurses' Health Study II[383] found that poor asthma control was associated with exposure to aldehydes, hypochlorite bleach, hydrogen peroxide, and enzymatic cleaners, but not with QACs. Our data provide clinical evidence supporting the findings of Gonzalez et al. [385] who established a significant relationship between exposure to QACs and an increased likelihood of physician-diagnosed asthma at work among hospital healthcare workers.

Strengths and limitations

The strength of this study was its multicenter design that allowed for gathering a large series of patients with QAC-induced OA confirmed by SIC after exclusion of subjects

concomitantly challenged with other potential respiratory sensitizers contained in cleaning products. Nevertheless, several limitations deserve further careful consideration. The major limitation of this study results from the limited number of subjects with available sputum samples. Nevertheless, the multivariate regression analysis confirmed that challenge exposure to QACs was the most significant determinant of the magnitude of the eosinophilic response to SIC in this cohort of subjects with OA induced by LMW agents, independently form potential confounders. In addition, the comparison of subjects with and without sputum samples suggests that there was no bias towards the performance of induced sputum in subjects with a higher likelihood of eosinophil recruitment in the airways. Indeed, Prince et al. [249] found that a lower baseline sputum eosinophil count, non-smoking, and a shorter exposure to the causal occupational agent were the only independent predictors for a greater eosinophilic response after SIC with occupational agents.

This study may also be criticized on the ground of its retrospective design and the use of different – though validated – methods for assessing NSBH and sputum cells. However, these between-center differences in procedures are unlikely to have affected the findings since the collection and interpretation of data were standardized for the whole cohort. Although SIC, the “reference” method for establishing a diagnosis of OA [398,399], is not thoroughly standardized, the centers participating in this cohort conformed with the main methodological requirements for safety and reliability issued by the Europen Respiratory Society, and airway responses to challenges exposures were interpreded using uniform criteria[284].

Another limitation arises from the lack of quantitative assessment of exposure to QACs at the workplace and during SIC. Dose-dependent bronchoconstriction induced by nebulized benzalkonium chloride (formerly used as a preservative in nebulizer solutions) has been described in asthmatics, although changes in NSBH and airway eosinophils have never been documented in such human inhalation studies with benzalkonium chloride[386,387,400]. On

the other hand, there is currently little information about exposure-response relationships since QACs have a low vapor pressure, and accurate sampling and analytical methods have only recently been developed in order detect very low levels of QACs in the air[401].

The potential role of irritants was not systematically investigated in this study because evidence-based and validated lists of substances that should be considered as respiratory irritants are currently lacking [402]. Epidemiological studies [378,379,381,382], surveillance programs [389,403], and case series [115] have most commonly related asthma in cleaners to respiratory irritants, such as bleach, ammonia; acids and oxydizers, although OA and work-exacerbated asthma could not be differentiated in these studies and the specific causal agents were not identified because SIC were generally not performed. The cleaning and/or disinfectant products that induced a positive SIC in the 22 subjects with QAC-induced OA in this cohort did not contain known respiratory irritants. However, four subjects in this cohort developed an asthmatic response during SIC with cleaning/disinfecting chemicals (i.e., peracetic acid in three subjects and sodium octyl sulfate in one subject) that failed to meet the structural requirements for being considered as respiratory sensitizers [389] (Table 11 in supplements). These findings further support previous reports of asthmatic reactions induced by peracetic/acetic acid mixtures [404,405] and the possible role of irritant ingredients in the development of cleaners' asthma. Interestingly, the positive SIC responses in our subjects were not associated with an increase in the level of NSBH or in sputum eosinophils, which is consistent with the findings of Sastre et al. [406] who performed inhalation challenges with bleach in 13 cleaners.

5.6 Conclusion

Despite its inherent limitations, this retrospective study provides further insight into the inflammatory mechanisms involved in the development of QAC-induced OA by demonstrating that the condition is associated with a highly eosinophilic airway response. The findings also highlight the respiratory sensitizing potential of these widely used biocide compounds. Awareness of this possibility may be relevant to the investigation of work-related asthma symptoms in workers involved in cleaning and disinfection tasks. Further prospective investigation of inflammatory markers and immunological mechanisms involved in QAC-induced OA is required to confirm and expand the findings of our retrospective study.

5.7 Supplementary

Assessment of respiratory sensitizing potential of cleaning ingredients

The respiratory sensitization potential of the cleaning and disinfecting products that elicited positive the SIC were assessed using two versions of a validated quantitative structure activity relationship (QSAR) model [126,407,408]. These QSAR models generate quantitative estimates of the probability that a low-molecular-weight (LMW) organic agent has respiratory sensitization potential based on its chemical structure (i.e. the "asthma hazard index" [HI]). The cut-point HI for discriminating potential respiratory sensitizers from inactive chemicals was 0.5 for the 2005 QSAR model (sensitivity 79%, specificity 93%)[408] and 0.39 for the 2015 model (sensitivity 90%, specificity 96%) [407]. The safety data sheets were also scrutinized for the presence of compounds previously documented as inducing asthma through inhalation challenges [372–374,376,404,405,409–423]. In addition to LMW agents, high-molecular weight enzymes were also considered as potential respiratory sensitizers [424,425] [26, 27], although the precise nature of enzymes was not provided by safety data sheets.

Table 9 Recruitment of the study population

Center	No. of yrs with IS during the 13-yr study period (2006-2018)	All agents		LMW agents		All cleaning agents		QAC	
		Positive SIC	IS n (%)	Positive SIC	IS n (%)	Positive SIC	IS n (%)	Positive SIC	IS n (%)
Barcelona, Spain	6	126	18 (14)	81	10 (12)	5	1 (20)	1	0
Bochum, Germany	6	8	5 (62)	7	4 (57)	0	0	0	0
Helsinki, Finland	3	264	24 (9)	107	8 (8)	2	0	0	0
Lodz, Poland	13	231	143 (62)	53	36 (68)	11	10 (91)	1	1
Strasbourg, France	6	58	17 (29)	32	7 (22)	11	2 (18)	9	3
Yvoir, Belgium	13	184	86 (47)	64	34 (53)	26	15 (58)	11	4
Total:		871	293 (34)	344	99 (29)	55	28 (51)	22	8 (36)

The rate of induced sputum was not significantly different in subjects who completed a SIC with QACs (8/22) as compared to other low-molecular-weight agents (71/289; p=0.220). The characteristics of subjects who completed an induced sputum assessment are compared with those who did are presented in Table 10. The subjects with available sputum analysis showed a higher median body mass index and were more frequently current or ex-smokers as compared to the subjects without sputum analysis. They also showed a higher median FVC (%predicted) and a lower FEV1/FVC ratio (%).

Table 10 Comparison of the baseline clinical and functional characteristics of subjects with and without induced sputum assessment

Characteristic	**Subjects without Induced sputum (n=232)**	**Subjects with induced sputum (n=79)**	***P*-value**
Age (yr)*	43 (34-52)	46 (39-53)	0.120
Sex (female)	126 (54.3)	51 (64.6)	0.117
Body mass index (kg/m^2)	26.2 (23.6-29.5)	28.3 (24.8-31.5)	**0.010**
Current/ex-smokers	113 (48.7)	50 (63.3)	**0.025**
Atopy[†]	100 (45.0)	28 (35.9)	0.184
Asthma pre-existing to the causal exposure	25 (10.8)	6 (7.6)	0.517
Duration of exposure before asthma onset (mo)*	76 [24-169]	105 [36-230]	0.273
Duration of symptomatic exposure (mo)*	25 [12-60]	36 [17-73]	0.129
Last exposure at work <1 week	62 (26.7)	27 (34.2)	0.249
Removal from work exposure at the time of SIC	120 (51.7)	42 (53.8)	0.745
Positive SIC to QAC	14 (6)	8 (8)	0.220
Coexisting conditions:			
Work-related rhinitis	135 (58.4)	42 (53.2)	0.432
Chronic rhinosinusitis	33 (14.3)	8 (10.1)	0.443
Work-related urticaria	21 (9.1)	5 (6.3)	0.638
Work-related contact dermatitis	34 (14.8)	8 (10.1)	0.346
Asthma treatment/severity at the time of SIC			0.095
Inhaled corticosteroid (daily dose, µg) [‡]	500 (0-1000)	500 (0-900)	0.153
Inhaled short-acting β_2-agonist ≥1/day	58 (25.0)	15 (19.0)	0.356
≥1 severe asthma exacerbation (last 12 mo)	66 (28.4)	20 (25.3)	0.663
Baseline spirometry			
FVC, % pred*	96 (86-106)	102 (90-111)	**0.026**
FEV1, % pred*	90 (81-99)	90 (76-100)	0.551
FEV1/FVC*	77 (72-82)	73 (67-80)	**0.002**
Baseline level of NSBH	(n=218)	(n=76)	0.679
Absent	61 (28.0)	20 (26.3)	
Mild	113 (51.8)	37 (48.7))	
Moderate-to-severe	44 (20.2)	19 (25.0)	
Post-challenge change in NSBH	(n=129)	(n=59)	
Pre/post-challenge NSBH ratio	1.6 (1.0-3.6)	2.3 (1.0-5.3)	0.120
Blood eosinophils	(n=94)	(n=76)	
Cells/µl*	222 (134-400)	240 (200-384)	0.554
Baseline FeNO	(n=65)	(n=50)	
ppb*	18 (10-31)	23 (15-32)	0.239
Pattern of bronchial response to SIC	(n=216) [§]	(n=67) [§]	0.164
Isolated early reaction	53 (24)	15 (22)	
Isolated late reaction	82 (38)	24 (36)	
Dual reaction	74 (34)	27 (40)	

Tableau 11Clinical, functional, and airway inflammatory markers in subjects with a positive SIC induced by cleaning products without identified respiratory sensitizer

Subject	Agent	Job/industry	Age (yr)	Sex (M/F)	Atopy	Baseline spirometry		Maximum fall in FEV_1 (% baseline)	NSBH		Sputum Eo (%)		Sputum Neu (%)		FeNO (ppb)	
						FEV_1 (%pred)	FEV_1/FVC (%)		Pre-BPT*	Pre/post ratio†	Pre-BPT	Post-BPT	Pre-BPT	Post-BPT	Pre-BPT	Post-BPT
Yv 790	Peracetic acid	Production worker/food industry	55	M	-	65	59	24	1.1	0.5	1	1	72	78	2	14
Yv 795	Peracetic acid	Cleaner/waste management	39	F	+	85	61	32	0.1	0.2	2	2	90	77	7	8
Ba 882	Peracetic acid	Auxilliary nurse	58	F	-	118	97	20	16.0	NA	NA	NA	NA	NA	NA	NA
Yv 672	Sodium octyl sulfate	Cleaner/food industry	49	F	-	78	63	26	0.7	0.7	2	2	28	76	8	9

Legend: *SIC specific inhalation Challenge*; *FeNO*, fractional exhaled nitric oxide; *FEV_1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *Na*, not available; *NSBH*, nonspecific bronchial hyperresponsiveness; *Neu*, neutrophils; *Eo*, eosinophils.

* Level of NSBH expressed as the concentration or dose of histamine/methacholine inducing a 20% fall in FEV_1 ($PC/PD_{20\%}$) expressed in mg/ml.

† A twofold or greater increase in the post-challenge level of NSBH (i.e. a pre/post $PC/PD_{20\%}$ ratio ≥2) was considered significant

6 Work-related dysphonia in subjects with sensitizer-induced occupational asthma is associated with neutrophilic airway inflammation

Nicolas Migueres,MD[1,2], Olivier Vandenplas MD, PhD[3], Jolanta Walusiak-Skorupa, MD, PhD[4], Xavier Munoz, MD, PhD[5], Hille Suojalehto, MD, PhD[6], Vera van Kampen, MD, PhD[7], Paola Mason, MD, PhD[8], Santiago Quirce, MD, PhD[9], Frédéric de Blay MD, PhD[1], on behalf of the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS)

Authors Affiliations:

1 Division of Pulmonology, Department of Chest Diseases, University Hospital of Strasbourg and Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France;

2 UMR 7357 Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie ICUBE, Strasbourg, France;

3 Service de Pneumologie, Centre Hospitalier Universitaire UCL Namur, Université Catholique de Louvain, Yvoir, Belgium;

4 Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Lodz, Poland;

5 Servei Pneumologia, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona and CIBER de Enfermedades Respiratorias (CIBERES), Barcelona, Spain;

6 Occcupational Health, Finnish Institute of Occupational Health, Helsinki, Finland

7 Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Ruhr University, Bochum, Germany;

8 Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padova, Padova, Italy;

9 Department of Allergy, La Paz University Hospital, IdiPAZ and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain;

* These authors contributed equally.

Contribution: This work was published *Clinical & Translational Allergy* as a letter. I was involved in data collection; I performed the data analysis and I wrote the first draft. Data managements was performed by Namur investigator (OV, CR).

6.1 French summary

La dysphonie est surreprésentée dans la population asthmatique, essentiellement d'origine fonctionnelle [207,208]. Il a été décrit des tableaux de dysfonction laryngée induite par le travail pouvant coexister avec un asthme professionnel [317]. L'objectif de cette étude était de caractériser sur le plan socio-démographique, clinique et inflammatoire les participants avec asthme professionnel qui rapportaient une dysphonie au travail. Cette étude a permis d'estimer que chez les patients issus de la base de données E-PHOCAS avec des données renseignées sur l'inflammation bronchique induite par expectoration induite au moment du test de provocation spécifique et sur la dysphonie au travail autodéclaré (n=341), 14,4% présentaient une dysphonie au travail. Les analyses multivariées par régression logistiques ont montré que la dysphonie au travail était indépendamment associée au genre féminin et à une inflammation neutrophilique. Malgré ses limitations inhérentes à son design rétrospectif, cette étude est la première à avoir montré une association entre la dysphonie au travail et l'inflammation neutrophilique. Cette étude souligne la nécessité de mener d'autres études prospectives à l'aide de questionnaires validés, d'une laryngoscopie et d'une analyse des expectorations induites afin d'explorer l'association entre le dysfonctionnement du larynx et l'inflammation neutrophile des voies respiratoires.

6.2 Main text

Vertigan et al. [208] recently highlighted the comorbid association between asthma and laryngeal dysfunction, although the pathophysiological mechanisms underlying this complex association remain largely uncertain [204]. It is widely acknowledged that laryngeal dysfunction, including vocal cord dysfunction, can be triggered by external stimuli, such exercise, strong odors and irritant exposures [204]. In this regard, workplace exposure to respiratory irritants has been reported as an important cause of the "work-related irritable larynx syndrome" [426].

We sought to assess the clinical characteristics and airway inflammatory processes associated with work-related dysphonia in a cohort of subjects with sensitizer-induced occupational asthma (OA) ascertained by a positive specific inhalation challenge (SIC). This retrospective study included 341 subjects identified among the multicenter European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) [299] who met the following eligibility criteria: 1) complete information on variables addressing asthma severity and control while exposed at work; 2) available information on self-reported dysphonia (i.e. hoarseness or loss of voice) at work; and 3) assessment of induced sputum cell counts at the time of the SIC procedure.

Forty-nine (14.4%) subjects experienced dysphonia while exposed at their workplace. The baseline clinical features and sputum cell counts of the subjects with and without dysphonia as well as the univariate associations with dysphonia are detailed in Table 12. A multivariable logistic regression analysis was conducted in order to identify the clinical and inflammatory characteristics that were associated with work-related dysphonia. The independent variables incorporated into these regression models included gender; sinusitis; high-level treatment at

work (i.e., Global Initiative for Asthma treatment step 4/5); poor asthma control at work (i.e., need for an inhaled short-acting ß2-agonist once or more a day); OA caused by a low- vs. a high-molecular-weight agent; as well as eosinophil and neutrophil sputum cell counts (expressed as % of total nonsquamous cells) (Table 13) .The multivariate logistic regression analysis revealed that female gender (odds ratio [OR], 2.04; 95% confidence interval [CI], 1.06-3.92; *P*=0.031) and a higher sputum neutrophil count (OR for each 5%-increase in neutrophil count, 1.09; 95% CI, 1.01-1.18; *P*=0.025) were significantly associated with a higher likelihood of work-related dysphonia (table 10). There was an association of borderline significance between dysphonia and high-level treatment (OR, 1.97; 95% CI, 0.97-3.95; *P*=0.057). Dysphonia showed a negative association with increased sputum eosinophil counts (OR, 0.41; 95% CI, 0.19-0.83; *P*=0.017).

Dysphonia is a main symptom of worked-associated irritable larynx syndrome (WILS) which has been defined as neuronal sensitization by a workplace trigger bringing about laryngeal dysfunction [426]. As recently described, neutrophil inflammation can regulate sensory neuron function, especially in chronic pain [427].

To our knowledge, our study is the first to describe a relationship between neutrophilic inflammation and work-related dysphonia.

We acknowledge the limitations inherent to the retrospective cross-sectional design of this study. The presence of dysphonia was not objectively documented through direct visualization of inappropriate laryngeal movement. In addition, dysphonia was not assessed during the SIC procedure implying that it was not possible to ascertain that the agent inducing the positive SIC response was also the cause of dysphonia at work.

Despite their inherent limitations, our findings suggest that airway neutrophilic inflammation could be involved in the development of work-related laryngeal dysfunction. This study

highlights the need for further prospective studies using validated questionnaires, laryngoscopy, and induced sputum analysis in order to explore the association between laryngeal dysfunction and neutrophilic airway inflammation.

Table 12 Univariate associations with self-reported dysphonia at work

Characteristics	**Missing values**	**Subjects without dysphonia at work** (n=292)	**Subjects with dysphonia at work** (n=49)	**OR (95% CI)**	***P* value**
Age, yr *	0	43 (34-51)	42 (38-52)	1.02 (0.99-1.05)	0.279
Sex, female	0	97 (33.2)	23 (46.9)	**1.78 (0.96-3.28)**	**0.065**
Body mass index ≥30 kg/m^2 *	0	82 (28.1)	15 (30.6)	1.13 (0.57-2.15)	0.717
Ex-smokers	0	83 (28.4)	10 (20.4)	0.66 (0.29-1.38)	0.286
Current smokers	0	62 (21.2)	12 (24.5)	1.05 (0.49-2.17)	0.890
Atopy[†]	4/0	146 (50.7)	28 (57.1)	1.30 (0.71-2.41)	0.405
Chronic rhinosinusitis	2/0	21 (7.2)	8 (16.3)	**2.50 (0.99-5.83)**	**0.041**
Exposure before symptom onset, mo*	2/0	108 (48-204)	150 (21-230)	1.00 (1.00-1.00)	0.218
Duration of asthma symptoms at work, mo*	3/0	36 (16-84)	33 (21-68)	1.00 (0.99-1.00)	0.522
Type of causal agent, LMW	0	191 (65.4)	25 (51.0)	**1.81 (0.98-3.33)**	**0.057**
Asthma treatment at work:					
Daily dose of ICS, µg*[‡]	0	500 (0-1000)	500 (0-1000)	1.00 (1.00-1.00)	0.827
High level treatment[§]	0	19 (6.5)	6 (12.2)	**2.12 (1.14-3.94)**	**0.017**
Poor asthma control while at work[π]	0	74 (25.3)	21 (42.9)	**2.21 (1.17-4.11)**	**0.013**
≥2 exacerbations last 12 mo at work	0	26 (8.9)	1 (2.0)	0.21 (0.01-1.04)	0.134
Baseline spirometry					
FVC, % pred*	0	101 (90-110)	103 (94-110)	1.01 (0.99-1.03)	0.610
FEV1, % pred*	0	90 (79-98)	91 (78-98)	1.01 (0.98-1.03)	0.602
FEV1/FVC, %*	0	74 (67-80)	75 (67-78)	1.00 (0.97-1.03)	0.981
Airflow obstruction[¶]	0	56 (19.2)	13 (26.5)	1.52 (0.73-3.00)	0.238
Baseline NSBH:	22/0				
Absent		56 (20.7)	11 (22.4)	1.11 (0.51-2.24)	0.787
Mild		139 (51.5)	27 (55.1)	1.16 (0.63-2.15)	0.641
Moderate/severe		75 (27.8)	11 (22.4)	0.75 (0.35-1.50)	0.440
Blood eosinophils, cells/µl*	58/10	280 (199-400)	249 (140-390)	1.00 (1.00-1.00)	0.335
Baseline FeNO, ppb*	184/10	22 (12-41)	22 (10-28)	**0.98 (0.96-1.00)**	**0.049**
Baseline sputum eosinophils:					
%*	0	2.0 (1.0-6.0)	1.2 (0.2-2.5)	**0.87 (0.77-0.95)**	**0.011**
≥3%		125 (42.8)	12 (24.5)	**0.43 (0.21-0.84)**	**0.018**
Baseline sputum neutrophils:					
%*	0	51.0 (36.0-70.0)	60.0 (48.2-78.5)	**1.02 (1.00-1.03)**	**0.017**
≥76%		57 (19.5)	15 (30.6)	**1.82 (0.91-3.52)**	**0.081**

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *NSBH*, nonspecific bronchial hyperresponsiveness; *LMW*, low-molecular-weight; *ICS*, inhaled corticosteroid; *SIC*, specific inhalation challenge. Data are presented as n (% of available data) unless otherwise specified. Bold indicates variable with univariate association demonstrating a p value under 0.1).

* Median value with interquartile range (IQR) within parentheses.

π Poor asthma control at work is defined as the use of SABA more than once a day

† Atopy defined by the presence of at least one positive skin prick test result to common allergens.
‡ Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.
§ High-level treatment defined as treatment step 4 or 5 of the Global Initiative for Asthma (http://www.ginasthma.org)
‖ Poor asthma control defined by the need for an inhaled short-acting □2-agonist once or more a day while exposed at work.
¶ Airflow obstruction defined by an FEV1 <80% predicted and an FEV1/FVC ratio <70%.

Table 13 Logistic multivariate model for dysphonia at work

Independent variables	**Dysphonia at work (n=49/341)**		
	OR	**(95% CI)**	***P* value**
Sex, female	2.04	(1.06-3.92)	**0.031**
Chronic rhinosinusitis			
Poor asthma control while at work$^{\pi}$	1.84	(0.91-3.71)	0.087
Type of causal agent, LMW			
High level treatment§	1.97	(0.97-3.95)	**0.057**
Eosinophil sputum cell counts ≥ 3%	0.41	(0.19-0.83)	**0.017**
Neutrophil sputum cell counts, 5% increase	1.05	(1.03-1.07)	**<0.001**

Legend :

π: Poor asthma control at work is defined as the use of SABA more than once a day

§: High-level treatment defined according to GINA as treatment step 4 or 5

The model included 338 patients; selection of variables was realised by a stepwise procedure based on Akaike information criterion.

7 Sputum Inflammatory Patterns are Associated with Distinct Clinical Characteristics in Subjects with Occupational Asthma Independently from the Causal Agent

Nicolas Migueres[1,2]*, Olivier Vandenplas[3]*, Jolanta Walusiak-Skorupa[4] , Marta Wiszniewska[4], Xavier Munoz[5], Christian Romero-Mesones[5], Hille Suojalehto[6], Irmeli Lindström[6], Vera van Kampen[7], Rolf Merget[7], Paola Mason[8], Piero Maestrelli[8], Joaquin Sastre[9], Santiago Quirce[10], Catherine Rifflart[3], Julien Godet[2,11], Frédéric de Blay[1], on behalf of the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS)

Authors Affiliations:

1 Division of Pulmonology, Department of Chest Diseases, University Hospital of Strasbourg and Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France;

2 UMR 7357 Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie ICUBE, Strasbourg, France;

3 Service de Pneumologie, Centre Hospitalier Universitaire UCL Namur, Université Catholique de Louvain, Yvoir, Belgium;

4 Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Lodz, Poland;

5 Servei Pneumologia, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona and CIBER de Enfermedades Respiratorias (CIBERES), Barcelona, Spain;

6 Occcupational Health, Finnish Institute of Occupational Health, Helsinki, Finland

7 Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Ruhr University, Bochum, Germany;

8 Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padova, Padova, Italy;

9 Department of Allergy, Fundacion Jimenez Dıaz, Universidad Autonoma de Madrid and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain;

10 Department of Allergy, La Paz University Hospital, IdiPAZ and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain;

11 Groupe Méthode Recherche Clinique, Pôle de Santé Publique, Strasbourg University, Strasbourg, France;

* These authors contributed equally.

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7.1 Abstract

Background: Clinical heterogeneity in sensitizer-induced occupational asthma (OA) and its relationship to airway inflammatory profiles remain poorly elucidated.

Objectives: To further characterize the interactions between induced sputum inflammatory patterns, asthma-related outcomes and the high- or low-molecular-weight category of causal agents in a large cohort of subjects with OA.

Methods: This multicenter, retrospective, cross-sectional study was conducted among 296 subjects with OA ascertained by a positive specific inhalation challenge who completed induced sputum assessment before and 24 hours after challenge exposure.

Results: Multivariate logistic regression analysis revealed that sputum eosinophilia ≥3% was significantly associated with a high dose of inhaled corticosteroid (odds ratio [95% confidence interval], 1.31 [1.11-1.55] for each 250-µg increment in daily dose), short-acting ß2-agonist use less than once a day (3.54 [1.82-7.00]), and the level of baseline nonspecific bronchial hyperresponsiveness (mild: 2.48 [1.21-5.08]); moderate/severe: 3.40 [1.44-8.29]). Sputum neutrophilia ≥76% was associated with age (1.06 [1.01-1.11]), male gender (3.34 [1.29-9.99]), absence of corticosteroid use (5.47 [2.09-15.16]), short-acting ß$_2$-agonist use once or more a day (4.09 [1.71-10.01]), ≥2 severe exacerbations during the last 12 months at work (4.22 [1.14-14.99]), and isolated early reactions during the SIC (4.45 [1.85-11.59]).

Conclusion: The findings indicate that sputum inflammatory patterns in subjects with OA are associated with distinct phenotypic characteristics and further highlight the differential effects of neutrophils and eosinophils on asthma-related outcomes. These associations between inflammatory patterns and clinical characteristics share broad similarities with what has been reported in nonoccupational asthma and are not related to the type of causal agent.

Abstract word count: 248 words

Keywords: Eosinophils; induced sputum; neutrophils; occupational asthma; phenotype.

7.2 French summary

Contexte : L'hétérogénéité clinique de l'asthme professionnel par sensibilisation et sa relation avec les profils inflammatoires des voies respiratoires restent mal élucidées.

Objectifs : Caractériser davantage les interactions entre les profils inflammatoires des expectorations induites, les résultats liés à l'asthme et la catégorie d'agents causale dans une grande cohorte de sujets souffrant d'asthme professionnel.

Méthodes : Cette étude transversale rétrospective multicentrique a été menée auprès de 296 sujets atteints d'asthme professionnel confirmés par un test de provocation bronchique spécifique (TPB) positif, qui ont bénéficié d'une évaluation des expectorations induites avant et 24 heures après l'exposition au test d'inhalation.

Résultats : L'analyse de régression logistique multivariée a révélé que l'éosinophilie des expectorations ≥3 % était significativement associée à une dose élevée de corticostéroïde inhalé (odds ratio [intervalle de confiance à 95 %], 1,31 [1,11-1. 55] pour chaque augmentation de 250 µg de la dose quotidienne), à l'utilisation d'un ß2-agoniste à courte durée d'action moins d'une fois par jour (3,54 [1,82-7,00]) et au niveau d'hyperréactivité bronchique non spécifique de base (légère : 2,48 [1,21-5,08]) ; modérée/sévère : 3,40 [1,44-8,29]). La neutrophilie des expectorations ≥76 % était associée à l'âge (1,06 [1,01-1,11]), au sexe masculin (3,34 [1,29-9,99]), à l'absence d'utilisation de corticostéroïdes (5,47 [2,09-15,16]), à l'utilisation de ß2-agonistes à courte durée d'action une fois ou plus par jour (4. 09 [1,71-10,01]), ≥2 exacerbations sévères au cours des 12 derniers mois au travail (4,22 [1,14-14,99]), et des réactions précoces isolées pendant le TPB (4,45 [1,85-11,59]).

Conclusion : Les résultats indiquent que les profils inflammatoires des expectorations induites chez les sujets atteints d'asthme professionnel sont associés à des caractéristiques phénotypiques distinctes et mettent en évidence les effets différentiels des neutrophiles et des

éosinophiles sur les caractéristiques cliniques lié à l'asthme. Ces associations entre les profils inflammatoires et les caractéristiques cliniques présentent de grandes similitudes avec ce qui a été rapporté dans l'asthme non professionnel et ne sont pas liées au type d'agent causal.

7.3 Introduction

Sensitizer-induced occupational asthma (OA), a distinguishable subset of adult asthma, is characterized by the *de novo* inception of asthma or the recurrence of previously quiescent asthma induced by immunologically mediated sensitization to specific agents at the workplace [428,429]. Workplace sensitizing agents are conventionally categorized into high-molecular-weight (HMW) (glycol)proteins from animal, vegetal or microbiological origin and low-molecular-weight (LMW) agents that include reactive chemicals, metals and wood dusts [428,429]. OA caused by HMW agents is associated with demonstrable specific IgE antibodies while LMW agents act as haptens, binding to endogenous proteins to initiate a specific immunologic response through mechanisms that remain largely unknown [285].

The noninvasive induced sputum technique allowed the identification of eosinophilic and noneosinophilic inflammatory patterns of asthma that are associated with different clinical phenotypes and are likely related to differences in underlying pathobiological pathways [224–226,228–230,232,234,235,253]. However, clinical heterogeneity in patients with OA and its relationship to sputum inflammatory profiles remain poorly elucidated. The available studies provided sparse and often discordant information pertaining to the relationships between eosinophilic inflammation and asthma outcomes[244–251]. In addition, most of these studies failed to specifically investigate the clinical and functional characteristics associated with sputum neutrophilia, although it has been suggested that OA induced by LMW agents may be associated with higher sputum neutrophilia compared to HMW agents [244,246,430].

This study aimed at further characterizing the relationships between sputum inflammatory patterns, asthma-related outcomes at the time of the diagnostic evaluation, and the type of causal agent in a large cohort of subjects with OA ascertained by a positive specific inhalation challenge (SIC).

7.4 Methods

7.4.1 Study Design and Population

This retrospective cross-sectional study was conducted among subjects with OA ascertained by a positive SIC completed between 2006 and 2018 in tertiary centers participating to the European network for the PHenotyping of OCcupational Asthma (E-PHOCAS) [280,294,299,388,431]. Eligible subjects for this analysis were those with complete information on key asthma outcomes (i.e. detailed medication and number of severe exacerbations) and induced sputum samples collected both before and 24 hours after the SIC procedure. Cohort recruitment is further detailed in the global method section. This report conformed to the Strengthening of the Reporting of Observational Studies in Epidemiology statement for cross-sectional studies (*www.strobe-statement.org*).

7.4.2 Ethics

This retrospective E-PHOCAS study was approved by the local Institutional Review Board of all participating sites as well as the "*Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé*" and the "*Commission Nationale de l'Informatique et des Libertés*".

7.4.3 Asthma Outcomes

Details of data collection and interpretation are given in the method section. Information on asthma-related outcomes while the subjects were exposed at work was used for this analysis. “Poor symptom control” was defined by the need for an inhaled short-acting ß2-agonist (SABA) once or more a day [349]. Severe asthma exacerbations and severe asthma were defined according to the European Respiratory Society/American Thoracic Society consensus guidelines [350].

7.4.4 Lung Function Assessments

Prechallenge spirometric values as well as the level of nonspecific bronchial hyperresponsiveness (NSBH) measured at baseline of the SIC procedure and 24 hours after challenge exposure were collected. NSBH was graded as “absent”, “mild”, or “moderate-to-severe” [280] according to the bronchoprovocation method used in each center (see method section). The methodology and interpretation of SIC is further described in the method section conformed with international recommendations [299].

7.4.5 Induced Sputum Assessment

Induced sputum was collected at baseline and 24 hours after the SIC. Detailed information on the methods used for the induction and analysis of sputum samples in participating centers is included in the method section. “Sputum eosinophilia” was defined by a sputum eosinophil count ≥3% while a sputum neutrophil count ≥76% was regarded as reflecting “sputum neutrophilia” [225]. Accordingly, the sputum inflammatory patterns were classified as “eosinophilic” (i.e., ≥3% eosinophils and <76% neutrophils); “neutrophilic” (i.e., neutrophils

≥76% and <3% eosinophils); “paucigranulocytic” (i.e., <3% eosinophils and <76% neutrophils); or “mixed granulocytic” (i.e., ≥76% neutrophils and ≥3% eosinophils).

In this study, we used sputum cell counts obtained 24 hours after the SIC as the primary outcome since a significant decrease in sputum eosinophil counts has been reported within two weeks after removal from exposure [432] and 173 of 296 (58.4%) subjects in this cohort were already removed from the causal exposure for more than one week at the time of the SIC procedure.

7.5 Data Analysis

Data are presented as the median (IQR) for continuous variables and percentages for categorical variables. Patients were compared between groups using the Fisher’s exact or chi-squared test for categorical variables and nonparametric tests for numerical variables. Pairwise comparisons between the 4 sputum inflammatory patterns to identify which groups differed from each other were corrected according to the Benjamini-Hochberg method for multiple comparisons.

Multivariable logistic regression analyses were conducted in order to identify the clinical and physiological characteristics associated with a ≥3% sputum eosinophil count or a ≥76% neutrophil count in samples collected 24 hours after challenge exposure. The multivariable analyses were carried out using a binomial generalized linear model with the most parsimonious models selected using a stepwise procedure based on the Akaike information criterion. The independent variables incorporated into these regression models included both sociodemographic characteristics (age, gender, smoking status, and body mass index ≥30 kg/m^2) and the variables with a *P*-value ≤0.1 in univariate comparisons. Missing values were

not imputed. Peripheral blood eosinophil counts and fractional exhaled nitric oxide (FeNO) were not included in the multivariate models because these biomarkers and sputum eosinophils are known to be interrelated [433] and reflect T2-high airway inflammation, and these data were missing in a substantial proportion of the subjects.

Sensitivity analyses were made by restricting the regressions to the pre-challenge (baseline) sputum eosinophil or neutrophil counts in the subjects who were still exposed to the offending agent within one week before the SIC procedure (n=123). Statistical analysis was performed using the R software version 3.4.1 (www.r-project.org, Vienna, Austria). A *P* value <0.05 was considered significant.

7.6 Result

7.6.1 Population

The study included 296 subjects with available sputum samples collected both before and 24 hours after the SIC procedure. The clinical and physiological characteristics of the subjects grouped according to their post-challenge sputum inflammatory pattern are presented in Tables 14 and 15. The majority (67.9%) of the subjects demonstrated a post-challenge eosinophilic pattern, whereas a paucigranulocytic, neutrophilic, or mixed granulocytic pattern was observed in 18.2%, 7.8%, and 6.1% of the cohort, respectively. The clinical and functional characteristics associated with the four sputum inflammatory patterns are compared in the supplements. The workplace agents causing OA in this cohort are detailed in Table 20(in supplementary).

Table 14 Demographic and clinical characteristics of the subjects according to their post-challenge sputum inflammatory pattern

Characteristic	Missing values	Eosinophilic pattern (n=201)	Mixed granulocytic pattern (n=18)	Neutrophilic pattern (n=23)	Paucigranulocytic pattern (n=54)	*P* value
Age, yr *	0	42 (34-51)	47 (40-54)	45 (37-54)	46 (34-51)	0.566
Sex, male	0	133 (66.2)	14 (77.8)	17 (73.9)	30 (55.6)	0.247
Body mass index, kg/m^2 *	0	27 (24-30)	26 (24-31)	27 (22-30)	28 (25-32)	0.413
Smoking status	0					0.711
Current-smoker	0	46 (22.9)	3 (16.7)	8 (34.8)	14 (25.9)	
Ex-smoker		54 (26.9)	6 (33.3)	7 (30.4)	17 (31.5)	
Never-smoker		101 (50.2)	9 (50.0)	8 (34.8)	23 (42.6)	
Atopy†	1/0/0/1	95 (47.5)	11 (61.1)	13 (56.5)	25 (47.2)	0.616
Chronic rhinosinusitis	1/0/0/1	13 (6.5)	3 (16.7)	3 (13.0)	7 (13.2)	0.119
Asthma pre-existing to the causal exposure	0	12 (6.0)	3 (16.7)	4 (17.4)	6 (11.1)	0.061
Duration of exposure before symptom onset, mo*	1/0/0/1	120 (60-216)	72 (28-240)	132 (50-228)	84 (25-204)	0.362
Duration of asthma symptoms at work, mo*	1/1/0/1	36 (18-86)	33 (13-72)	36 (16-48)	24 (15-60)	0.246
Interval since last work exposure, mo*	1/0/0/0	3.0 (0.03-11.2)	6.5 (0.1-16.8)	5.0 (0.1-14.5)	2.8 (0.1-10.8)	0.388
Type of causal agent, HMW	0	139 (69.2)	8 (44.4)	16 (69.6)	25 (46.3)	**0.005**
Work-related rhinitis	0	164 (81.6)	14 (77.8)	14 (60.9)	37 (68.5)	**0.042**
Asthma treatment at work						
ICS use	0	160 (79.6)	10 (55.6)	11 (47.8)	36 (66.7)	**0.001**
Daily dose of ICS, µg*‡	0	500 (400-1000)	325 (0-1000)	0 (0-1000)	500 (0-605)	0.052
Long-acting β2-agonist	0	155 (77.1)	10 (55.6)	10 (43.5)	32 (59.3)	**0.001**
Leukotriene receptor antagonist	1/1/1/0	50 (25.0)	3 (17.6)	2 (9.1)	9 (16.7)	0.262
Poor asthma control (SABA ≥ once a day) at work	0	45 (22.4)	5 (27.8)	16 (69.6)	13 (24.1)	**<0.001**
≥2 severe exacerbations last 12 mo at work	0	16 (8.0)	4 (22.2)	2 (8.7)	1 (1.9)	**0.045**
Severe asthma at work§	0	35 (17.4)	5 (27.8)	5 (21.7)	8 (14.8)	0.549

Legend: HMW, high-molecular-weight; *ICS,* inhaled corticosteroid; *SABA,* short-acting β2-agonist. The sputum inflammatory patterns were characterized as "eosinophilic" (i.e. ≥3% eosinophils and <76% neutrophils); "neutrophilic" (i.e. ≥76% and <3% eosinophils); "paucigranulocytic" (i.e. <3% eosinophils and <76% neutrophils); and "mixed granulocytic" (i.e. ≥76% neutrophils and ≥3% eosinophils. Data are presented as n (% of available data) unless otherwise specified. Bold indicates statistical significance (*P*<0.05).

* Median value with interquartile range (IQR) within parentheses.

† Atopy defined by the presence of at least one positive skin prick test result to common allergens.

‡ Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

§ Multidimensional definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society guidelines [350]

Table 15 Functional characteristics and markers of airway inflammation according to the post-challenge sputum inflammatory pattern

Characteristic	Missing values	Eosinophilic pattern (n=201)	Mixed granulocytic pattern (n=18)	Neutrophilic pattern (n=23)	Paucigranulocytic pattern (n=54)	*P* value
Baseline spirometry						
FVC, % pred*	0	102 (91-110)	100 (91-104)	105 (91-112)	102 (91-109)	0.928
FEV1, % pred*	0	90 (78-97)	88 (72-92)	85 (73-96)	93 (84-101)	0.251
FEV1/FVC, %*	0	74 (66-80)	71 (66-77)	73 (64-78)	78 (72-81)	**0.024**
Baseline level of NSBH†	14/2/0/4					
Absent		30 (16.0)	2 (12.5)	4 (17.4)	18 (36.0)	**0.020**
Mild		99 (52.9)	10 (62.5)	12 (52.2)	23 (46.0)	0.703
Moderate-to-severe		58 (31.0)	4 (25.0)	7 (30.4)	9 (18.0)	0.324
Post-SIC change in NSBH	71/6/2/11					
Pre/post-SIC NSBH ratio*		2.33 (1.40-4.64)	2.40 (1.31-4.05)	1.48 (1.00-2.67)	2.58 (1.00-6.70)	0.293
Maximum fall in FEV1, % baseline*	0	24 (19-33)	29 (24-37)	22 (18-27)	22 (19-27)	0.193
Pattern of bronchial response to SIC	12/4/4/4					**0.008**
Isolated immediate reaction		67 (35.4)	9 (64.3)	13 (68.4)	17 (34.0)	
Late reaction‡		122 (64.6)	5 (35.7)	6 (31.6)	33 (66.0)	
Blood eosinophils	33/3/4/14					
Cells/µl*		300 (200-428)	203 (150-300)	211 (144-305)	200 (100-291)	**<0.001**
>300/µl		93 (55.4)	5 (33.3)	6 (31.6)	9 (22.5)	**<0.001**
Baseline FeNO, ppb*	136/6/5/25	26 (14-39)	24 (16-61)	12 (7-26)	18 (9-28)	0.123
Post-SIC change in FeNO	139/6/5/31					
ppb*§		20 (6-46)	11 (4-29)	2 (0-12)	2 (0-12)	**0.001**
>17.5 ppb§		35 (56.5)	3 (25.0)	3 (16.7)	4 (17.4)	**0.001**
Baseline sputum inflammatory pattern	0					ND
Eosinophilic		95 (47.3)	0	1 (4.3)	7 (13.0)	
Neutrophilic		18 (9.0)	6 (33.3)	14 (60.9)	6 (11.1)	
Mixed granulocytic		10 (5.0)	4 (22.2)	3 (13.0)	1 (1.9)	
Paucigranulocytic		78 (38.8)	8 (44.4)	5 (21.7)	40 (74.1)	

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *ND*, not done; *NSBH*, nonspecific bronchial hyperresponsiveness; *SIC,* specific inhalation challenge. The sputum inflammatory patterns were characterized as "eosinophilic" (i.e. ≥3% eosinophils and <76% neutrophils); "neutrophilic" (i.e. ≥76% and <3% eosinophils); "paucigranulocytic" (i.e. <3% eosinophils and <76% neutrophils); and "mixed granulocytic" (i.e. ≥76% neutrophils and ≥3% eosinophils. Data are presented as n (% of available data) unless otherwise specified. Bold indicates statistical significance (*P*<0.05).

* Median value with interquartile range (IQR) within parentheses.

† See Table S1 for the grading of nonspecific bronchial hyperresponsiveness.

‡ Late asthmatic reactions including isolated late and dual reactions.

§ Difference from baseline value

7.6.2 Determinants of Sputum Eosinophilia

The clinical and physiological characteristics of the subjects with (n=219) and without (n=77) a post-challenge sputum eosinophil count ≥3% and the univariate associations with post-challenge sputum eosinophilia are detailed in Table 16. The multivariate logistic regression analysis (Table 17; multivariate model 1) revealed that a high dose of ICS (odds ratio, 1.31; 95% confidence interval [CI], 1.11-1.55 for each 250-µg increment in daily dose of ICS; *P*=0.002), SABA use less than once a day (odds ratio, 3.54; 95% CI, 1.82-7.00; *P*<0.001), as well as mild (odds ratio, 2.48; 95% CI, 1.21-5.08; *P*=0.012) and moderate-to-severe NSBH (odds ratio, 3.40; 95% CI, 1.44-8.29; *P*=0.006) were significant predictors of a post-challenge sputum eosinophilia.

The multivariate analysis of the pre-challenge sputum eosinophil count ≥3% restricted to subjects still exposed at work within one week of the SIC procedure (n=123) (Table 13) also identified a high dose of ICS, SABA use less than once a day and the level of NSBH as the main factors associated with sputum eosinophilia. Univariate associations with pre-challenge sputum eosinophilia are detailed in Table 21(in supplementary).

Table 16 Univariate associations with pre-challenge sputum eosinophilia and neutrophilia among subjects still exposed at work

Characteristics	**Pre-SIC sputum eosinophilia ≥3%*** (n=60)		**Pre-SIC sputum neutrophils ≥76%** [a] (n=30)	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Age, yr [a]	0.99 (0.95-1.03)	0.675	1.01 (0.97-1.06)	0.588
Sex, male	0.86 (0.40-1.85)	0.705	1.72 (0.69-4.73)	0.260
Smoking habit				
Never smoker	-		-	
Ex-smoker	0.64 (0.27-1.46)	0.290	1.06 (0.40-2.73)	0.903
Current smoker	0.68 (0.27-1.68)	0.404	0.88 (0.28-2.49)	0.810
Body mass index, ≥30 kg/m^2 [a]	1.07 (0.47-2.44)	0.878	0.40 (0.11-1.14)	0.114
Atopy [b]	0.94 (0.46-1.92)	0.866	1.09 (0.48-2.52)	0.831
Chronic rhinosinusitis	0.87 (0.27-2.79)	0.817	0.91 (0.19-3.24)	0.893
Childhood asthma	0.68 (0.17-2.50)	0.564	2.23 (0.54-8.42)	0.240
Exposure before symptom onset, mo [a]	1.00 (1.00-1.00)	0.631	1.00 (1.00-1.01)	0.373
Duration of asthma symptoms at work, mo	1.00 (0.99-1.01)	0.830	1.00 (0.99-1.01)	0.784
HMW causal agent (vs. LMW agent)	1.73 (0.80-3.81)	0.169	1.64 (0.66-4.52)	0.306
Associated work-related rhinitis	0.62 (0.25-1.52)	0.299	2.62 (0.82-11.74)	0.142
Asthma treatment at work:				
ICS use	2.07 (0.91-4.94)	0.090	0.31 (0.13-0.77)	**0.010**
Daily dose of ICS, µg [a, c]	1.27 (1.05-1.57)	**0.019**	0.92 (0.73-1.13)	0.479
SABA ≥ 1/day at work	0.97 (0.42-2.25)	0.950	1.56 (0.60-3.89)	0.343
≥2 severe exacerbations last 12 mo at work	3.39 (0.75-23.80)	0.145	0.42 (0.02-2.52)	0.431
Severe asthma at work[d]	2.00 (0.74-5.75)	0.178	1.54 (0.50-4.36)	0.430
Baseline spirometry:				
FVC, % pred [a]	1.00 (0.98-1.02)	0.967	1.01 (0.99-1.04)	0.301
FEV1, % pred [a]	2.03 (0.93-4.56)	0.081	0.84 (0.32-2.07)	0.719
FEV1/FVC, %	0.94 (0.91-0.98)	**0.004**	1.01 (0.97-1.05	0.664
Baseline level of NSBH [e]				
Absent	-		-	
Mild	3.61 (1.03-16.97)	0.063	0.31 (0.09-1.11)	0.067
Moderate-to-severe	8.33 (2.26-40.87)	**0.003**	0.92 (0.28-3.17	0.887
Pre/post-SIC NSBH ratio >2 [a]	0.60 (0.24-1.50)	0.274	0.65 (0.24-1.83)	0.407
Maximum fall in FEV1 during the SIC, % baseline [a]	1.28 (0.96-1.76)	0.105	1.30 (0.94-1.80)	0.107
Isolated immediate vs late reaction [f]	0.73 (0.34-1.53)	0.403	1.79 (0.71-4.66)	0.220
Baseline blood eosinophil count,				
cells/µl [a]	1.00 (1.00-1.01)	**0.030**	1.00 (1.00-1.00)	0.329
>300/µl	1.64 (0.76-3.57)	0.212	0.64 (0.25-1.57	0.335
Baseline FeNO, ppb [a]	1.00 (0.99-1.02)	0.616	1.00 (0.97-1.02)	0.754
Post-SIC change in FeNO, ppb [a]	1.00 (0.98-1.03)	0.664	1.00 (0.98-1.02)	0.929

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *HMW*, high-molecular-weight; *ICS*, inhaled corticosteroid; *LMW*, low-molecular-weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OR*, odds ratio; *SABA*, short-acting β_2-agonist*; SIC*, specific inhalation challenge. Univariate analyses of prechallenge sputum cells were performed among 123 subjects who were still exposed at work at the time of the evaluation (within two weeks). Data are presented as n (% of available data) unless otherwise specified. Bold indicates statistical significance (P<0.05). Bold indicates statistical significance (*P*<0.05)

[a] Median value with interquartile range (IQR) within parentheses.

[b] Atopy defined by the presence of at least one positive skin prick test result to common allergens.

[c] Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

[d] Multidimensional definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society guidelines [350]

[e] See methods for the grading of nonspecific bronchial hyperresponsiveness.

[f] The SIC was considered positive based on a significant increase in the post-challenge level of NSBH (i.e., pre/post ratio >2) while the changes in FEV1 remained <15% in 24 subjects.

Table 17 Multivariate models for sputum eosinophilia

Independent variables	Post-challenge sputum eosinophils ≥3% (n=219/296)			Pre-challenge sputum eosinophils ≥3% [a] (n=60/123)		
	OR	**(95% CI)**	***P* value**	**OR**	**(95% CI)**	***P* value**
Final model 1:						
Causal agent, HMW vs. LMW	1.68	(0.87-3.24)	0.119	2.43	(0.86-7.47)	0.104
Work-related rhinitis	1.69	(0.83-3.41)	0.146	0.35	(0.10-1.15)	0.095
ICS daily dose, per 250 µg	1.31	(1.11-1.55)	**0.002**	1.36	(1.09-1.76)	**0.010**
SABA ≥1/day	0.28	(0.14-0.55)	**<0.001**	0.28	(0.09-0.84)	**0.028**
Level of baseline NSBH, vs. no NSBH[b]						
Mild	2.48	(1.21-5.08)	**0.012**	3.64	(0.97-18.01)	0.075
Moderate/severe	3.40	(1.44-8.29)	**0.006**	7.74	(1.85-41.72)	**0.008**
Final model 2:						
HMW causal agent *plus* rhinitis at work	2.78	(1.55-5.08)	**0.001**	1.07	(0.45-2.53)	0.881
ICS daily dose, per 250 µg	1.32	(1.12-1.57)	**0.001**	1.30	(1.05-1.67)	**0.024**
SABA ≥1/day	0.28	(0.14-0.54)	**<0.001**	0.32	(0.10-0.93)	**0.042**
Level of baseline NSBH, vs. no NSBH[b]						
Mild	2.57	(1.25-5.29)	**0.010**	3.54	(0.97-17.10)	0.075
Moderate/severe	3.54	(1.48-8.73)	**0.005**	8.83	(2.18-46.42)	**0.004**

Legend: *HMW,* high-molecular-weight; *ICS*, inhaled corticosteroid; *LMW*, low-molecular-weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OR*, odds ratio; *SABA,* inhaled short-acting β_2-agonist.

The multivariate model 1 incorportated HMW causal agents vs. LMW agents and work-related rhinitis (yes/no) as independent variables, whereas model 2 included only the association between a HMW agent and work-related rhinitis. The multivariate models included 275 subjects for the analysis of post-challenge sputum eosinophilia and 111 subjects for pre-challenge sputum eosinophilia. Bold indicates statistical significance (P<0.05).

[a] Multivariate analysis conducted among 123 subjects still at work at the time of assessment (see Table 18 in the supplement materials for univariate analyses of pre-challenge neutrophilia).

[b] See Table methods the grading of nonspecific bronchial hyperresponsiveness.

7.6.3 Determinants of Sputum Neutrophilia

Table 18 provides the characteristics of subjects with (n=41) and without (n=255) a post-challenge sputum neutrophil count ≥76% and the univariate associations with post-challenge sputum neutrophilia. The multivariate analysis (Table 19) showed that sputum neutrophilia was significantly associated with older age (odds ratio for a 1-yr increase, 1.06; 95% CI, 1.01-1.11; P=0.014), male gender (odds ratio, 3.34; 95% CI, 1.29-9.99; P=0.019), absence of ICS

use (odds ratio, 5.47; 95% CI, 2.09-15.16; *P*<0.001), poor asthma control (odds ratio, 4.09; 95% CI, 1.71-10.0; *P*=0.024), a history of two or more severe exacerbations during the last 12 months while exposed at work (odds ratio, 4.22; 95%CI, 1.14-14.9; *P*=0.025), and the development of isolated immediate reactions during the SIC (odds ratio, 4.45; 95% CI, 1.85-11.59; *P*=0.001).

Table 21 in the Supplements provides the univariate associations for pre-challenge sputum neutrophili count ≥76% among the 123 subjects still exposed at work. The multivariate analysis of pre-challenge sputum neutrophilia retained only the absence of ICS use (odds ratio, 5.09; 95% CI, 1.82-15.22; *P*=0.002) as a significant factor associated with sputum neutrophilia (Table 19).

Table 18 Univariate associations with post-challenge sputum neutrophilia

Characteristics	Missing values	Sputum neutrophils ≥76% (n=41)	Sputum neutrophils <76% (n=255)	Univariate analysis	
				OR (95% CI)	*P* value
Age, yr [a]	0/0	46 (37-55)	43 (34, 51)	1.02 (0.99-1.06)	0.154
Sex, male	0/0	31 (76)	163 (64)	1.75 (0.85-3.91)	0.148
Smoking habit	0/0				
Never smoker		17 (41)	124 (49)	-	
Ex-smoker		13 (32)	71 (28)	1.34 (0.60-2.90)	0.467
Current smoker		11 (27)	60 (24)	1.34 (0.58-3.00)	0.487
Body mass index, ≥30 kg/m^2 [a]	0/0	12 (29)	76 (30)	0.97 (0.46-1.97)	0.944
Atopy [b]	0/2	24 (59)	120 (47)	1.56 (0.81-3.10)	0.189
Chronic rhinosinusitis		6 (15)	20 (8)	2.00 (0.69-5.06)	0.166
Childhood asthma	0/0	7 (17)	18 (7)	2.71 (0.99-6.74)	**0.038**
Exposure before symptom onset, mo [a]	0/2	120 (48-240)	120 (48, 204)	1.00 (1.00-1.00)	0.560
Duration of asthma symptoms at work, mo [a]	1/2	36 (12-52)	36 (16, 84)	1.00 (0.99-1.00)	0.738
Interval since last work exposure, mo [a]	0/1	5 (0-15)	3 (0, 11)219	1.19 (0.90-1.59)	0.225
HMW causal agent (vs. LMW agent)	0/0	24 (59)	164 (64)	0.78 (0.40-1.55)	0.476
Associated work-related rhinitis	0/0	28 (68)	201 (79)	0.58 (0.29-1.22)	0.138
Asthma treatment at work:	0/0				
ICS use		21 (51)	196 (77)	0.32 (0.16-0.62)	**0.001**
Daily dose of ICS, µg [a,c]		250 (0-1000)	500 (250, 1000)	0.90 (0.74-1.07)	0.249
SABA ≥ 1/day at work		21 (51)	58 (23)	3.57 (1.81-7.08)	**<0.001**
≥2 severe exacerbations last 12 mo at work	0/0	6 (15)	17 (7)	2.40 (0.82-6.22)	0.085
Baseline spirometry:	0/0				
FVC, % pred [a]		103 (91-110)	102 (91, 110)	1.00 (0.98-1.02)	0.906
FEV1, % pred [a]		88 (73-96)	90 (79, 98)	1.59 (0.78-3.14)	0.192
FEV1/FVC <0.70		16 (39)	89 (35)	0.97 (0.94-1.0)	0.163
Baseline level of NSBH	2/18				
Absent		6 (15)	48 (20)	-	
Mild		22 (56)	122 (51)	1.44 (0.58-4.11)	0.456
Moderate-to-severe				1.31 (0.47-4.04)	0.615
Pre/post-SIC NSBH ratio >2	8/82	15 (45)	114 (66)	0.43 (0.20-0.92)	**0.029**
Maximum fall in FEV1, % baseline [a]				0.94 (0.68-1.26)	0.693
Isolated immediate reaction during the SIC [d]	8/16	22 (67)	84 (35)	3.69 (1.74-8.25)	**0.001**
Baseline blood eosinophil count:	7/47				
cells/µl [a]		207 (145-300)	292 (200, 400)	1.00 (1.00-1.00)	0.065
>300/µl		11 (32)	102 (49)	0.50 (0.22-1.05)	0.074
Baseline FeNO, ppb [a]	11/161	16 (9-38)	23 (10, 37)	1.00 (0.98-1.01)	0.717
Post-SIC change in FeNO, ppb [a]	11/170	4 (1-15)	13 (2, 38)	0.99 (0.97-1.00)	0.154

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *HMW,* high-molecular-weight; *ICS,* inhaled corticosteroid; *LMW*, low-molecular-weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OR,* odds ratio; *SABA,* short-acting β_2-agonist*; SIC,* specific inhalation challenge. Data are presented as n (% of available data) unless otherwise specified. Bold indicates statistical significance (P<0.05).

[a] Median value with interquartile range within parentheses.

[b] Atopy defined by the presence of at least one positive skin prick test result to common allergens.

[c] Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent

[d] The SIC was considered positive based on a significant increase in the post-challenge level of NSBH (i.e., pre/post ratio >2) while the changes

Table 19 Multivariate models for sputum neutrophilia

Independent variables	Post-challenge sputum neutrophils ≥76% (n=41/296)			Pre-challenge sputum neutrophils ≥76%[a] (n=30/123)		
	OR	**(95% CI)**	***P* value**	**OR**	**(95% CI)**	***P* value**
Final model:						
Age, yr	1.06	(1.01-1.11)	**0.014**	1.05	(1.00-1.11)	0.073
Gender, male	3.34	(1.29-9.99)	**0.019**	2.75	(0.92-9.77)	0.089
Body mass index ≥30 kg/m^2	-			0.32	(0.08-1.00)	0.064
No ICS use	5.47	2.09-15.16	**<0.001**	5.09	(1.82-15.22)	**0.002**
SABA ≥1/day	4.09	(1.71-10.01)	**0.024**	-		
Severe exacerbation, ≥2 last 12 mo at work	4.22	(1.14-14.99)	**0.025**	-		
Isolated early reaction vs. late reaction	4.45	(1.85-11.59)	**0.001**	-		

Legend: *ICS*, inhaled corticosteroid; *OR*, odds ratio; *SABA,* short-acting β_2-agonist.

The multivariate models included 271 subjects for the analysis of post-challenge sputum neutrophilia and 123 subjects for pre-challenge sputum neutrophilia among subjects still exposed at work. Bold indicates statistical significance (*P*<0.05).

[a] Multivariate analysis conducted among subjects still at work at the time of assessment (see Table 18 in the supplement materials for univariate analyses of pre-challenge neutrophilia).

7.6.4 Associations between Causal Agents and Sputum Inflammatory Patterns

Although Table 14 indicates that HMW agents were more frequently involved in subjects who showed an eosinophilic pattern, exposure to a HMW agent was not retained as a significant determinant for sputum eosinophilia in the multivariate analysis (Table 17; multivariate model 1). Interestingly, however, the presence of work-related rhinitis in subjects exposed to a HMW agent (Table 17; model 2) was significantly associated with a post-challenge sputum eosinophil count ≥3% (odds ratio, 2.78; 95% CI, 1.55-5.0; *P*=0.001), while the dose of ICS, SABA use less than once a day, and NSBH remained significant.

Of note, HMW agents were involved in 24 of 41 (58.5%) subjects with a post-challenge sputum neutrophilia ≥76% (*see* Table 22 in the Supplement). Flour was the predominant agent, accounting for 19 of 24 cases related to HMW agents. Interestingly, IgE-mediated sensitization to flour was documented by skin-prick testing and/or the determination of specific IgE antibodies in 20 of these 24 subjects with HMW-induced sputum neutrophilia.

7.7 Discussion

This large cohort study is, to our knowledge, the first that comprehensively characterizes the clinical and functional characteristics associated with neutrophilic and eosinophilic airway inflammation in OA.

The multivariate analysis revealed that post-challenge sputum neutrophilia ≥76% was significantly and independently associated with older age, poorer asthma control, more frequent severe asthma exacerbations, and low ICS use during exposure at work. These findings are consistent with studies conducted in general adult asthma populations that documented associations between neutrophilic airway inflammation and age [224,228,230], more severe disease and poorer asthma control[229,232,235], although sputum neutrophilia was not associated with poor lung function in our cohort of subjects with OA [224,228].

While data for this cohort indicated that neutrophilic OA showed phenotypic similarities with nonoccupational asthma, slight differences were also detected compared with the findings of studies conducted in general adult asthma populations. Male sex was associated with an increase likelihood of sputum neutrophilia, while a female preponderance has been reported in asthma in the general population [230]. Another intriguing observation was the association between post-challenge sputum neutrophilia and isolated immediate asthmatic reactions that has to our knowledge not been described after inhalation challenges with common inhalant allergens in subjects with allergic asthma [434].

The mechanisms underlying neutrophilic airway inflammation and its role in asthma remain an area of intense research [435,436]. It is currently acknowledged that neutrophilic inflammation may reflect innate immune responses to environmental triggers, such as ozone, diesel exhaust particulates and bacterial endotoxin (lipopolysaccharide) which have been documented as inducing an increase in sputum neutrophils. Remarkably, a substantial proportion (58.5%) of the subjects with sputum neutrophilia in our cohort were challenged with HMW protein agents,

mainly flour, while IgE-mediated sensitization to these HMW agents was documented in the vast majority (83.3%) of these subjects (table 18 (in the supplement). This observation is consistent with an earlier study that demonstrated neutrophilic airway inflammation in subjects with OA who developed predominantly immediate asthmatic reaction after challenge exposure to cereal grain dust extracts [437]. Sputum neutrophilia, either isolated or combined with sputum eosinophilia, has also been documented in 11% of subjects with allergic asthma after an inhalation challenge with nonoccupational inhalant allergens [438]. Endotoxins that likely "contaminate" allergen extracts and HMW workplace agents may promote neutrophilic inflammation via Toll-like receptor-4 signalling [439]. Collectively, these data further indicate that a HMW agent is capable of initiating either type 2 (T2), non-T2, or mixed immune responses. Further prospective investigations should be performed to determine whether the elicitation of T2 vs. non-T2 immune responses is affected by host-related (e.g., airway microbiome) and environmental factors (e.g., endotoxin) that interact with HMW occupational agents.

Most patients with OA (67.9%) demonstrated a post-challenge sputum eosinophilic pattern which is consistent with what has been described in individuals exposed to common inhalant allergens [438]. However, there is limited information about the determinants of eosinophilic airway inflammation and its relationship with the clinical and functional phenotypes in subjects with OA. Previous studies in OA related sputum eosinophilia to more severe disease at the time of the diagnostic work-up. This was associated with worse quality of life [244] and more frequent use of ICS [244] compared to noneosinophilic OA in subjects sensitized to LMW agents. In this OA cohort, the eosinophilic pattern was associated with higher dose of ICS and mild disease activity in terms of symptoms, exacerbations, and airway obstruction. Therefore, eosinophilic OA shared common features with the "eosinophilic inflammation-predominant" cluster described by Haldar et al. [253] in a secondary care cohort of adult asthmatics. These

findings further highlight the possible dissociation between eosinophilic inflammation and asthma symptoms [253].

Previous studies conducted in limited series of subjects with OA predominantly exposed to LMW agents, such as isocyanates or Western red cedar, found that sputum eosinophil counts correlated positively with the degree of baseline airflow obstruction [244,245] and a higher level of NSBH [244], although sputum eosinophilia failed to correlate with NSBH in some of these studies [245,246]. Our large cohort study provides definitive evidence that sputum eosinophilia is strongly associated with a higher level of baseline NSBH, consistent with findings in general asthma populations [225,228,440]. In contrast, we failed to document a relationship between sputum eosinophilia and the baseline FEV1/FVC ratio or the FEV1 [244,245]. Such relationships might have been blunted in this OA cohort because spirometry was measured at baseline of the SIC while 58.4% of the subjects were no longer exposed to the offending workplace. Nevertheless, analyzing pre-challenge sputum eosinophils among subjects still exposed at work within one week of the SIC procedure further confirmed that sputum eosinophilia was not associated with the level of airflow obstruction (Table 13 and Table 17 (in supplements)).

Remarkably, this cohort study demonstrates that both sputum eosinophilia and neutrophilia develop independently of the molecular weight category of the causal agents, which is in line with the study by Prince et al [249] , who compared the changes in sputum cell during SICs with HMW and LMW agents. Nevertheless, in our cohort, HMW agents were significantly associated with postchallenge sputum eosinophilia only when there was coexisting work-related rhinitis. This finding is consistent with the observation that nasal exposure to inhalant allergens enhance eosinophil recruitment into the lower airways in persons with allergic rhinitis [441,442] and may account for the discordant information on the patterns of airway inflammation induced

by HMW and LMW agents reports by previous studies that not take account the co-occurrence of occupational rhinitis [244,246,250].

The findings of this study challenge the traditional concept of categorizing the agents causing OA into HMW and LMW, presuming implicitly that they act through different underlying pathophysiological mechanisms. Our data provide convincing evidence that the molecular weight category of the causal agent does not determine the pattern of airway inflammation, although HMW and LMW agents are associated with distinct clinical characteristics, especially those pertaining to IgE- related clinical features. Indeed, a previous analysis conducted in a larger sample of the E-PHOCAS cohort that did not take into account sputum inflammatory data demonstrated that OA caused by HMW agents was characterized by a higher rate of work-related rhinitis, atopy, isolated early asthmatic reactions, and a greater postchallenge increase in FeNO compared with OA induced by LMW agents [280]. In addition, recent data from this E-PHOCAS cohort revealed pathophysiological heterogeneity among LMW agents. Compared with other LMW agents, acrylate-induced OA [294] was characterized by factors that are similar to those of OA caused by HMW agents (ie, concomitant work-related rhinitis and a greater postchallenge increase in FeNO), while OA caused by quaternary ammonium compounds was associated with a more marked eosinophilic response than other LMW agents [431]. However, the number of patients with OA caused by most of the LMW agents was too limited in this cohort to allow further comparison of the clinical and inflammatory characteristics between the various types of LMW agents.

The major strengths of this study were the homogeneous diagnostic criteria used to identify OA and the multicentric design, which allowed for the recruitment of a large cohort of patients evaluated by SIC and induced sputum. Nevertheless, several limitations deserve further consideration. The major potential limitation of the study resulted from the use of sputum cell

counts obtained 24 hours after challenge exposure to the causal agents as a surrogate for the airway inflammatory profile of patients while exposed at work. However, the multivariate analysis of baseline sputum eosinophilia restricted to those who were still exposed at work at the time of the diagnosis yielded results similar to those of the analysis of postchallenge sputum eosinophilia among the whole cohort (Table 17). In contrast, low ICS use was the only determinant of baseline neutrophilia among patients still at work, while postchallenge neutrophilia was significantly associated with demographic and clinical characteristics (Table 19), suggesting that neutrophil counts after acute exposure to occupational agents may be more clinically relevant than those recorded during long-term exposure at work. Although the multicenter design of this study enabled the recruitment of a large cohort of patients who had undergone assessment of induced sputum, the number of patients with sputum neutrophilia was still low and might not have enabled us to fully capture the influence of potential environmental and host factors and distinguish accurately between neutrophilia alone (n=23) and neutrophilia in combination with sputum eosinophilia (ie, mixed granulocytic pattern, n=18) (see Table 10 and Supplements).

The retrospective cross-sectional design did not allow us to determine whether the persistence of sputum eosinophilia despite ICS treatment might result from suboptimal doses of ICS, poor treatment adherence, or mishandling of inhaler devices. Nevertheless, functional stability was established by monitoring FEV1 on the control day before challenge exposure to the causal agents. Likewise, we were not able to evaluate whether the higher rates of poor asthma control and severe exacerbations in patients with sputum neutrophilia were related to the neutrophilic inflammation per se or to the less frequent use of ICS.

Another limitation of this retrospective multicenter study resulted from the use of slightly different methods to induce and process sputum samples and the lack of quality control. There is conflicting information as to whether variations in the methods may have impacted the

differential sputum cell counts [443], although using different nebulizers and saline concentrations does not affect sputum cell counts [444,445]. There were also between-center differences in the bronchoprovocation methods used to assess the level of NSBH; nevertheless, the interpretation of results was standardized for the whole cohort

7.8 Conclusion

This large cohort study indicates that sputum inflammatory patterns are associated with distinct clinical phenotypes of OA. However, the associations between sputum inflammatory patterns and phenotypic characteristics in OA share broad similarities with data reported for nonoccupational asthma. These findings may improve our understanding of the pathophysiological mechanisms involved in OA and enhance precision medicine. Nevertheless, the question of whether inflammatory patterns have an impact on the long- term outcome of the disease should be further addressed in prospective studies. In addition, our data provide definitive evidence that a sensitizing occupational agent can induce OA through different inflammatory pathways, independently of its HMW or LMW category.

7.9 Supplementary

Clinical Characteristics Associated with Sputum Inflammatory Patterns

Paucigranulocytic pattern. The paucigranulocytic pattern showed the highest median FEV1/FVC ratio, but the difference was significant only when compared with the eosinophilic (*P*=0.036) patterns. Subjects with a paucigranulocytic pattern showed less often significant NSBH (66.7% vs. 85.1% vs. 88.9% vs. 82.6% for paucigranulocytic, eosinophilic, mixed and neutrophilic patterns, respectively; across-group *P*=0.020).

Neutrophilic pattern. The neutrophilic pattern was characterized by the highest rate (69.6%) of poor asthma control compared with the eosinophilic (22.4%; *P*=0.016), mixed granulocytic (27.8%; *P*=0.059), and paucigranulocytic (24.1%; *P*=0.016) patterns. Subjects with a neutrophilic pattern had the lowest rate of treatment with ICS (47.8%), but the difference reached statistical significance only when compared with the eosinophilic pattern (79.6%; *P*=0.016). They showed a higher rate (68.4%) of isolated immediate reactions after challenge exposure to the causal agent compared with the eosinophilic (35.4%; *P*=0.036) and paucigranulocytic patterns (34.0%; *P*=0.061), but this rate was similar to that recorded in the mixed granulocytic pattern (64.3%).

Mixed granulocytic pattern. The mixed granulocytic pattern exhibited the highest proportion (22.2%) of subjects who experienced two or more severe exacerbations during the last 12 months at work, but this proportion did not differ significantly from the paucigranulocytic (1.7%; *P*=0.059), eosinophilic (8.0%; *P*=0.178), and neutrophilic (8.7%; *P*=0.609) patterns.

Eosinophilic pattern. Subjects with a post-challenge eosinophilic pattern showed a trend toward the highest use of ICS while exposed at work. These subjects were treated with an ICS significantly more frequently (79.6%) than those with a neutrophilic (47.8%; *P*=0.016), but their ICS use did not differ from the subjects with a mixed granulocytic (55.6%, *P*=0.119) or a paucigranulocytic pattern (66.7%; *P*=0.178). The eosinophilic pattern was associated with the highest baseline blood eosinophil count and

the greatest post-challenge increase in FeNO compared to the neutrophilic (P=0.088 and P=0.016, respectively) and paucigranulocytic patterns (P=0.016 and P=0.016, respectively), but these indices were not significantly different from the mixed granulocytic pattern.

Table 20 Workplace agents causing occupational asthma

High-molecular-weight agents	**n (%) ***	**Low-molecular-weight agents**	**n (%) ***
Flour/grains	148 (50.2)	Isocyanates	28 (9.5)
Latex	11 (3.7)	Various cleaning products/disinfectants†	17 (5.8)
Enzymes	5 (1.7)	Metals	11 (3.7)
Storage mites	4 (1.4)	Wood dusts	10 (3.4)
Fish/seafood	4 (1.4)	Persulfate salts	10 (3.4)
Cow dander	2 (0.7)	Quaternary ammonium compounds†	7 (2.4)
Rodents	2 (0.7)	Acrylate compounds	6 (2.0)
Molds	2 (0.7)	Welding fumes	5 (1.7)
Insects (parasitoid wasps)	1 (0.3)	Metal working fluids	3 (1.1)
Various plant-derived products	6 (2.0)	Amines	3 (1.1)
Various animals and derived products	3 (1.1)	Colophony	2 (0.7)
		Resins/glues/paints (NOS)	2 (0.7)
		Various low-molecular-weight agents	4 (1.4)
Total:	188 (63.5)	Total:	108 (36.5)

Legend:

* Expressed as % of total identified agents (n=296).
† Cleaning products contained mixtures of various chemicals; seven subjects were challenged only with quaternary ammonium compounds [431]

Table 21 Univariate associations with pre-challenge sputum eosinophilia and neutrophilia among subjects still exposed at work

Characteristics	**Pre-SIC sputum eosinophilia ≥3%*** (n=60)		**Pre-SIC sputum neutrophils ≥76%** [a] (n=30)	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Age, yr [a]	0.99 (0.95-1.03)	0.675	1.01 (0.97-1.06)	0.588
Sex, male	0.86 (0.40-1.85)	0.705	1.72 (0.69-4.73)	0.260
Smoking habit				
Never smoker	-		-	
Ex-smoker	0.64 (0.27-1.46)	0.290	1.06 (0.40-2.73)	0.903
Current smoker	0.68 (0.27-1.68)	0.404	0.88 (0.28-2.49)	0.810
Body mass index, ≥30 kg/m^2 [a]	1.07 (0.47-2.44)	0.878	0.40 (0.11-1.14)	0.114
Atopy [b]	0.94 (0.46-1.92)	0.866	1.09 (0.48-2.52)	0.831
Chronic rhinosinusitis	0.87 (0.27-2.79)	0.817	0.91 (0.19-3.24)	0.893
Childhood asthma	0.68 (0.17-2.50)	0.564	2.23 (0.54-8.42)	0.240
Exposure before symptom onset, mo [a]	1.00 (1.00-1.00)	0.631	1.00 (1.00-1.01)	0.373
Duration of asthma symptoms at work, mo	1.00 (0.99-1.01)	0.830	1.00 (0.99-1.01)	0.784
HMW causal agent (vs. LMW agent)	1.73 (0.80-3.81)	0.169	1.64 (0.66-4.52)	0.306
Associated work-related rhinitis	0.62 (0.25-1.52)	0.299	2.62 (0.82-11.74)	0.142
Asthma treatment at work:				
ICS use	2.07 (0.91-4.94)	0.090	0.31 (0.13-0.77)	**0.010**
Daily dose of ICS, µg [a, c]	1.27 (1.05-1.57)	**0.019**	0.92 (0.73-1.13)	0.479
SABA ≥ 1/day at work	0.97 (0.42-2.25)	0.950	1.56 (0.60-3.89)	0.343
≥2 severe exacerbations last 12 mo at work	3.39 (0.75-23.80)	0.145	0.42 (0.02-2.52)	0.431
Severe asthma at work[d]	2.00 (0.74-5.75)	0.178	1.54 (0.50-4.36)	0.430
Baseline spirometry:				
FVC, % pred [a]	1.00 (0.98-1.02)	0.967	1.01 (0.99-1.04)	0.301
FEV1, % pred [a]	2.03 (0.93-4.56)	0.081	0.84 (0.32-2.07)	0.719
FEV1/FVC, %	0.94 (0.91-0.98)	**0.004**	1.01 (0.97-1.05	0.664
Baseline level of NSBH [e]				
Absent	-		-	
Mild	3.61 (1.03-16.97)	0.063	0.31 (0.09-1.11)	0.067
Moderate-to-severe	8.33 (2.26-40.87)	**0.003**	0.92 (0.28-3.17	0.887
Pre/post-SIC NSBH ratio >2 [a]	0.60 (0.24-1.50)	0.274	0.65 (0.24-1.83)	0.407
Maximum fall in FEV1 during the SIC, % baseline [a]	1.28 (0.96-1.76)	0.105	1.30 (0.94-1.80)	0.107
Isolated immediate vs late reaction [f]	0.73 (0.34-1.53)	0.403	1.79 (0.71-4.66)	0.220
Baseline blood eosinophil count,				
cells/µl [a]	1.00 (1.00-1.01)	**0.030**	1.00 (1.00-1.00)	0.329
>300/µl	1.64 (0.76-3.57)	0.212	0.64 (0.25-1.57	0.335
Baseline FeNO, ppb [a]	1.00 (0.99-1.02)	0.616	1.00 (0.97-1.02)	0.754
Post-SIC change in FeNO, ppb [a]	1.00 (0.98-1.03)	0.664	1.00 (0.98-1.02)	0.929

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV1*, forced expiratory volume in one-second; *FVC*, forced vital capacity; *HMW*, high-molecular-weight; *ICS*, inhaled corticosteroid; *LMW*, low-molecular-weight; *NSBH*, nonspecific bronchial hyperresponsiveness; *OR*, odds ratio; *SABA*, short-acting β_2-agonist*; SIC*, specific inhalation challenge. Univariate analyses of prechallenge sputum cells were performed among 123 subjects who were still exposed at work at the time of the evaluation (within two weeks). Data are presented as n (% of available data) unless otherwise specified. Bold indicates statistical significance (P<0.05). Bold indicates statistical significance (*P*<0.05)

[a] Median value with interquartile range (IQR) within parentheses.

[b] Atopy defined by the presence of at least one positive skin prick test result to common allergens.

[c] Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

[d] Multidimensional definition of severe asthma adapted from the European Respiratory Society/American Thoracic Society guidelines [350].

[e] See Methods for the grading of nonspecific bronchial hyperresponsiveness.

[f] The SIC was considered positive based on a significant increase in the post-challenge level of NSBH (i.e., pre/post ratio >2) while the changes in FEV1 remained <15% in 24 subjects.

Table 22 Workplace agents involved in post-challenge sputum neutrophilia

Causal agents	**Post-challenge neutrophilic pattern** (N=23)			**Post-challenge mixed granulocytic pattern** (N=18)		
	N	**Positive SPT**	**Positive sIgE**	**N**	**Positive SPT**	**Positive sIgE**
High-molecular-weight agents:						
Flour/grains	14	11/14	11/14	5	5/5	5/5
Latex	1	1/1	0/1	1	1/1	1/1
Chamomile flowers	1	1/1	1/1	-	-	-
Tomato flowers and leaves	-	-	-	1	1/1	1/1
Animal fur (fox, mink)	-	-	-	1	ND	ND
Low-molecular-weight agnents:						
Isocyanates	2	0/1	0/1	3	ND	1/2
Cleaning products[a]	2	ND	ND	4	ND	ND
Metal dust containing cobalt	1	0/1	ND	-	-	-
Metal working fluids	1	ND	ND	1	ND	ND
Persulfates salts	1	ND	ND	1	ND	ND
Drug (erythromycine)	-	-	-	1	0/1	ND

Legend: *ND*, not done; *sIgE*, specific IgE antibody assessment; *SPT*, skin-prick test.

[a] Cleaning agents contained various chemical compounds, including an amine surfactant and peracetic acid in the two subjects with a neutrophilic pattern, peracetic acid in one subject with a mixed granulocytic pattern, and multiple potential sensitizers in the other 3 subjects with a mixed granulocytic pattern.

8 Irritant occupational exposure and specific-IgE sensitization in the EGEA cohort

Nicolas Migueres, MD,[1,2,3], Orianne Dumas,PhD, [4], Anne Boudier,MSc,[1] Jean Bousquet,MD,PhD,[5] Raphaëlle Varraso,PhD,[6] Rachel Nadif,PhD[4] Rudolf Valenta,MD,PhD[7,8] Frederic de Blay,MD,PhD[2,9], Nicole Le Moual,PhD,[4]Valérie Siroux,PhD[1]

Affiliation:
1: University Grenoble Alpes, Inserm U1209, CNRS UMR5309, Team of Environmental Epidemiology Applied to the Development and Respiratory Health, Institute for Advanced Biosciences, IAB, Grenoble 38000, France
2 : Service de Pneumologie et Allergologie, Pôle de Pathologie Thoracique, University Hospital of Strasbourg, Strasbourg, France ;
3 : UMR 7357 Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie ICUBE, Strasbourg University, Strasbourg, France
4 : CESP-U 1018 Inserm, Villejuif, France
5 : University Hospital Montpellier,Montpellier, France
6 : UVSQ, UMR-S 1168 - Saint-Quentin-en- Yvelines, France
7 : Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
8 : Karl Landsteiner University, Krems, Austria
9 : EA 3072 Fédération de Médecine translationnelle, Strasbourg University, Strasbourg, France

Keyword: occupational irritant exposure, epidemiology, asthma

Contribution: This work is in preparation. I performed the data analysis and I wrote the first draft.

Abbreviations used:

HMW : High molecular weight
HDM: House dust mite
LMW : Low molecular weight
sIgE: Allergen-specific IgE
OAsJEM: occupational asthma-specific job-exposure matrix

Abstract

Introduction: Biological mechanisms involved in the irritant-induces asthma remain partly unknown, in particular the role of IgE sensitization has not been thoroughly studied. We investigated the association between irritant occupational exposure (IOE) and IgE sensitization according to asthma status (no asthma, childhood- and adult-onset asthma).

Methods: The study is based on 924 adult participants to the first follow-up of the Epidemiological study of the Genetics and Environment of Asthma (EGEA2) and 271 participants recruited as children at baseline (EGEA1) with occupational data at follow-up. Allergen-specific IgE (sIgE) sensitization to 162 allergen molecules was assessed by microarray technology. Lifetime IOE was evaluated by the occupational asthma-specific job-exposure matrix (OAsJEM). Adjusted regression models estimated cross-sectional and longitudinal associations between OEI and the number and cluster-based profiles of IgE-reactive allergens.

Results: 30.7% and 22.8% participants were exposed to medium and high IOE. Among adult-onset asthma participants, IOE were associated to a lower risk of exhibiting house dust mite predominant sIgE sensitization (OR (95%CI) = 0.32 (0.12-0.86); p= 0.02) and high IOE tended to be associated with both lower risk of pollen/animals type of sIgE sensitization (OR:0.37(0.13-1.02); p=0.06) and fewer sIgE sensitization (adjusted mean ratio: 0.63 (0.39-1.02) p=0.061). No association was observed in non-asthmatics and childhood-onset asthma participants. No longitudinal association was observed between sIgE sensitization in childhood and IOE in adulthood.

Conclusion: Irritant occupational exposure tended to be associated with fewer sIgE sensitization, irrespectively of the type of sensitization, only in adult-onset asthma. Large longitudinal researches are needed to clarify whether IOE induce lower sensitization or elicit asthma in individuals with few allergens sensitization profile.

Résumé

Introduction : Les mécanismes biologiques impliqués dans l'asthme induit par les irritants restent en partie inconnus, en particulier le rôle de la sensibilisation aux IgE n'a pas fait l'objet d'études approfondies. Nous avons étudié l'association entre l'exposition professionnelle aux irritants (EPI) et la sensibilisation aux IgE en fonction du statut de l'asthme (pas d'asthme, asthme de l'enfance et asthme de l'adulte).

Méthodes : L'étude est basée sur 924 participants adultes au premier suivi de l'étude épidémiologique de la génétique et de l'environnement de l'asthme (EGEA2) et 271 participants recrutés comme enfants au début de l'étude (EGEA1) avec des données professionnelles lors du suivi. La sensibilisation aux IgE spécifiques à l'allergène (sIgE) à 162 molécules d'allergènes a été évaluée par la technologie de micro array. L'exposition professionnelle vie entière a été évaluée à l'aide de la matrice emploi-exposition spécifique à l'asthme professionnel (OAsJEM). Des modèles de régression ajustés ont permis d'estimer les associations transversales et longitudinales entre l'EPI et le nombre et les profils de sensibilisation IgE spécifique

Résultats : 30,7 % et 22,8 % des participants ont été exposés à des EPI moyennes et élevées. Parmi les participants souffrant d'asthme à l'âge adulte, les EPI étaient associées à un risque plus faible de présenter une sensibilisation sIgE à prédominance d'acariens (OR (95%CI) = 0,32 (0,12-0,86) ; p= 0. 02) et il existant une tendance entre une EPI élevée et le fait d'être associée à la fois à un risque plus faible de sensibilisation aux sIgE de type pollen/animaux (OR:0,37(0,13-1,02) ; p=0,06) et à un moindre nombre de sensibilisation sIgE (rapport de moyenne ajusté : 0,63 (0,39-1,02) p=0,061). Aucune association n'a été observée chez les non-asthmatiques et les participants souffrant d'asthme apparu dans l'enfance. Aucune

association longitudinale n'a été observée entre la sensibilisation aux sIgE dans l'enfance et les EIP à l'âge adulte.

Conclusion : L'exposition aux irritants professionnel tend à être associée à une moindre sensibilisation aux sIgE, quel que soit le type de sensibilisation, uniquement dans le cas de l'asthme apparu à l'âge adulte. Des recherches longitudinales de grande envergure sont nécessaires pour déterminer si l'exposition professionnelle irritante induit une moindre sensibilisation ou déclenche un asthme chez les personnes présentant un profil de sensibilisation à peu d'allergènes.

8.1 Introduction

Occupational exposures have been estimated to induce up to 16% of asthma cases [19]. Irritant induced asthma is an underestimated cause of occupational asthma whose mechanisms, presumed to be non-immunological in nature, remain poorly understood [269,277,339].

Relationship between IgE sensitization and occupational irritant exposure (OIE) has not been thoroughly investigated. Lilienberg and coworkers described, in a longitudinal populational based study, that non atopic participants exposed to low molecular weight agents (LMW) and irritants were at higher risk to develop asthma than atopic participants [343]. However, atopy was defined as a positive answer to 'Do you have hay fever or any other nasal allergy?'; definition which might lack sensitivity. Results from a cross sectional study indicated that non atopic asthma, defined by total IgE <100 U/ml and negative Phadiatop test result (<0.35 PAU/L), was significantly associated with exposure to LMW agents including industrial cleaning agents and irritant peaks [347]. Conflicted results have been observed between exposure to cleaning agent and IgE sensitization [346,446–450]. In previous studies, atopy has been treated as an all-or-nothing process in terms of binary logic, which is a limited approach.

The development of allergen microarray technology, capable of simultaneously detecting the antibody reactivity profiles to a wide range of allergen molecules, has improved the characterization of allergic sensitization [68,363]. Cluster-based statistical approaches, able to summarized the wealth of information provided by allergen microarray technology across allergen-specific IgE sensitization profiles, provide a comprehensive approach for advancing our understanding of atopy [68,451]. Recently, profiles of allergen-specific IgE (sIgE) sensitization have been associated with respiratory outcomes and have been shown to be stable

from childhood to adulthood [68]. Using micro array technology to assess sIgE sensitization may help us understanding the relationship between OIE and sIgE sensitization.

Measuring the association between OEI and IgE sensitization could be challenging because of a possible healthy hire effect; sensitized participants during childhood could avoid jobs associated with OIE [367,368]. Whether sIgE sensitization could induce a healthy hire effect is not yet elucidated [452–454].

Taking advantage of the detailed sIgE characterization of participants to the Epidemiological study of the Genetics and Environement of Asthma (EGEA), we aimed to investigate the association between irritant occupational exposures and allergen specific IgE sensitization assessed by micro-array technology in asthmatic and non-asthmatic subjects, considering a possible healthy worker effect.

8.2 Methods

8.2.1 Study design setting and participants:

The Epidemiological Study on the Genetics and Environment of Asthma (EGEA) is a French cohort including participants with asthma recruited in chest clinics, their first-degree relatives and population-based participants (from electoral rolls for adults and recruited in surgery department for children). In total, 1,440 adults and 607 children were recruited from 1991 to 1995 (EGEA1) through self-completed questionnaires and had a complete examination including pulmonary function tests and blood sample. Approximately 12 years later, this population was invited for a follow-up (EGEA2) and 1601 participants (77.1% of the original cohort +58 new family members) participated in another complete examination. Ethical approval was obtained for both surveys from the local ethics committees (Cochin Royal Hospital, Paris for EGEA1 and Necker-Enfants Malades Hospital, Paris for EGEA2).

Allergen-specific IgE (sIgE) to microarrayed allergen molecules were measured in 333 children at EGEA1 and 933 subjects EGEA2. The selection of sera was independent of total IgE, respiratory symptoms and lung function levels, limiting any risk for a selection bias. Participants with data available for sIgE, asthma status, age at asthma onset, and occupational exposure, were included in the analysis (Figure. 13).

The cross-sectional association between IOE and sIgE sensitization was assessed among adult participants to EGEA2. The longitudinal association between childhood sIgE sensitization and IOE during adulthood was assessed in EGEA1 participants with follow up data at EGEA2.

8.2.2 Allergen-specific IgE (sIgE) measurements:

IgE reactivity to microarrayed allergen molecules was quantitatively determined in anonymized samples with the MeDALL-chip by a blinded operator [363]. The MeDALL-chip comprises 162 allergen components including aero- and food allergen components. Allergen-specific IgE data were discretized using a binary threshold (positive >0.30 ISU) [365]. Profile of sIgE sensitization were defined by cluster analysis (see statistical analysis). The number of positive sIgE responses to respiratory allergens was defined by the IgE reactivity to 63 clinically relevant respiratory allergenic molecules (table 8 in general method).

8.2.3 Occupational exposure measurements:

EGEA participants completed a questionnaire about their occupational history which was coded by an expert according to the International Standard Classification of Occupation (ISCO)-88.

We applied the occupational asthma-specific job-exposure matrix (OAsJEM) [360] which estimates exposure to 30 occupational agents divided into 3 groups (table 7): HMW sensitizers, LMW sensitizers and irritants. Each exposure was evaluated in 3 levels: high (high probability

of exposure and moderate to high intensity), medium (low to moderate probability or low intensity of exposure), no/low (unlikely to be exposed; low probability and low intensity).
We studied lifetime occupational exposures to irritant. Each exposure was assessed in two categories (ever exposed vs. never exposed) or three categories (medium exposure, high exposure, never exposed).

8.2.4 Asthma definition

Asthma was defined by positive answer to the questions ‘‘Have you ever had attacks of breathlessness at rest with wheezing?’’ or ‘‘Have you ever had asthma attacks?’’, or being recruited as an asthma case in chest clinics at EGEA1. Subjects were considered as having children-onset asthma if the age of onset was below 16 and adult-onset asthma if the age of onset was above or equal 16 years old. Asthma severity was defined following the principle of the Global Initiative for Asthma (GINA) 2002 guideline [455]. Subjects were identified with “moderate-severe asthma” if they reported at least one asthma attack in the past 12 months and either 1) ICS treatment use in the past 12 months; 2) an asthma score ≥3; or 3) an asthma score = 2 and use of asthma treatment other than ICS. The other subjects were classified as “past or mild” asthma.

8.3 Statistical analysis

Baseline characteristics are presented as frequencies for categorical date, medians and IQR for non-normally distributed continuous data and mean and SD for normally distributed continuous data. Participants were compared between groups using the Fisher’s exact or chi-squared test for categorical variables and the nonparametric Kruskal-Wallis test for numerical variables. To identify sIgE sensitization profiles, Latent Class Analysis (LCA), a data-driven approach, was

applied on IgE-reactivity as in previous work [68]. However, the number of allergens used and the number of clusters were different in order to avoid missing data and to have enough individuals in each group to allow multivariate analysis. Indeed, LCA was applied to 37 *a priori* most relevant respiratory allergens, being recognized by enough participants (n>10), without bringing redundant information, without missing data. LCA was applied on IgE reactivity of these 37 allergens. Each participant was assigned to the latent cluster (here defined as sIgE sensitization profile) for which he/she had the highest membership probability. The criteria for selecting the number of clusters were: 1) the model with the lowest Bayesian information criterion [369] 2) the model with number of observation for each cluster greater than 10% of the total number of observations. The cross-sectional association between the level of occupational irritant exposure at EGEA 2 and the number of sIgE sensitization at EGEA2 according to asthma status was assessed by negative binomial analysis. This regression models the ratio ("adjusted mean ration" aMR) of the mean number of positive sIgE sensitization between participants exposed and not exposed to OIE, independently to adjustments variables. For example, an aMR of 0.5 for OIE means that participants exposed to irritants exhibit a mean number of positive sIgE twice as low than those who are not exposed. The cross-sectional association of occupation irritant exposure at EGEA 2 with the type of sensitization at EGEA2 according to asthma status was assessed by multinomial logistic regression. Adjustment was performed for age, sex, smoking habit and early country living. A sensitivity analysis was performed after exclusion of participants co-exposed to HMW agents. Longitudinal analysis of the association of sIgE sensitization at EGEA 1 with OIE at EGEA 2 was assessed by multinomial logistic regression and logistic regression with adjustment for age and sex and father diploma level. The missing data rate was respectively 0.9 and 3.5% for the cross sectional and the longitudinal analysis. Multiple imputation by chained equation was undergone for missing data using mice package in R. Statistical analysis was performed using the R software

version 4.3.2 (*www.r-project.org*, Vienna, Austria). Interpretation of statistical tests was based on examining both ORs and aMR magnitude, their 95% CI, and precise p values (not whether P values are above or below 0.05) [456,457]

8.4 Result

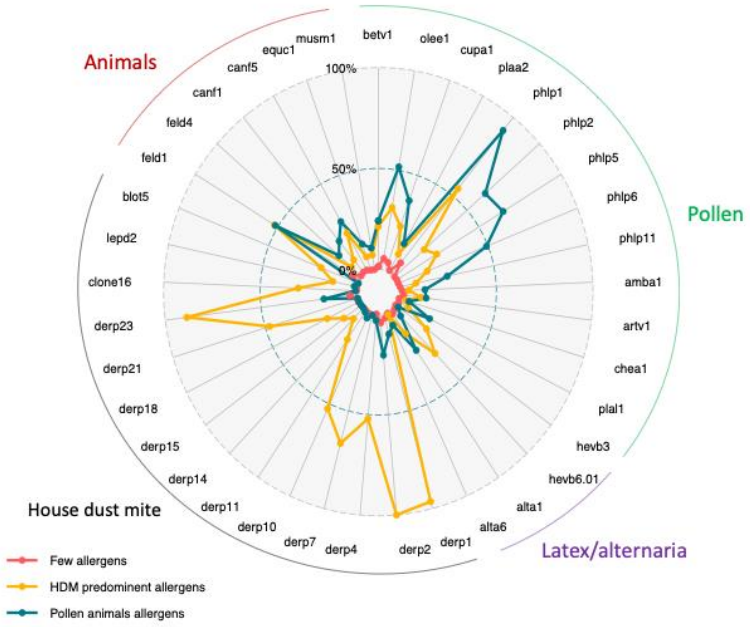

Figure 16 Radar chart showing IgE-reactivity probabilities to individual respiratory allergen molecules for each sensitization profile identified by the latent class analysis at EGEA2

Participants' characteristics at EGEA 2

Analyses were conducted on 924 participants, including 50% with asthma. Among participants with asthma, 39% reported onset of asthma in adulthood (table 23). The mean age of participant was 42.3 years (SD ±16.3). Compared to adult-onset asthma and non-asthmatic participants, childhood-onset asthmatic participants were younger, more frequently female and experienced more often early country living. Regarding OIE 30.7% and 22.9 % of the population were respectively exposed at medium and high level of exposure, without significant differences according to asthma status.

Three distinct sIgE sensitization profiles were identified at EGEA 2, each accounting for more than 10% of the population. They were described by displaying the IgE-reactivity probability to individual respiratory allergen molecules for each profile using a radar chart (Figure 16). The mean ± SD of the maximum posterior probabilities of belonging to the assigned profile was high (0.98 ± 0.07). The sIgE sensitization profiles identified were characterized as follows:

- (1) Few allergens molecules profile, (57.6 % of the population) mostly composed of participants with no sIgE sensitization to respiratory allergens (68%) or very few sIgE sensitization.
- (2) Pollen/animals allergen molecules profile, (19% of the population), was mainly composed of participant with positive sIgE to timothy grass, tree allergens, cat, dog and horse allergens.
- (3) House dust mite (HDM) allergens predominant profile (23.4% of the population).

According to asthma status, the median number of sIgE sensitization and the profile of sensitization differed. Adult-onset asthma participant and non-asthmatic participants elicited predominantly few allergens profile of sensitization (which represented respectively 60.2% and 79.6% of the sensitization profile in these groups). Children onset asthma participants exhibited mostly HDM predominant allergens profile and had the highest median number of sIgE sensitization compared to the other groups (median (IQR): 8.00 (4.25,13.0).

Table 23 EGEA 2 patients characteristics

Characteristic	Overall, **N = 924**[1]	Childhood asthma, **N = 282**[1]	adult onset asthma, **N = 181**[1]	no asthma, **N = 461**[1]	Overall p value
Age	42.27 (16.31)	33.36 (13.94)	50.39 (13.96)	44.53 (16.09)	<0.01
Sex, female	483 (52.3%)	126 (44.7%)	98 (54.1%)	259 (56.2%)	<0.01
Smoking habits					<0.01
No smoker	463 (50.1%)	136 (48.2%)	96 (53.0%)	231 (50.1%)	
Ex-smoker	252 (27.3%)	61 (21.6%)	59 (32.6%)	132 (28.6%)	
Current smoker	209 (22.6%)	85 (30.1%)	26 (14.4%)	98 (21.3%)	
Asthma severity					
Mild	354 (83.9%)	228 (87.0%)	126 (78.8%)	-	
Moderate to severe	68 (16.1%)	34 (13.0%)	34 (21.3%)	-	
Unknown	*41*	*20*	*21*		
Early country living	112 (12.9%)	18 (6.6%)	29 (17.6%)	65 (15.1%)	<0.01
Unknown	*57*	*11*	*16*	*30*	
Occupational exposure to irritant					0.07
Never	429 (46.4%)	140 (49.6%)	90 (49.7%)	199 (43.2%)	
Medium exposure	284 (30.7%)	91 (32.3%)	45 (24.9%)	148 (32.1%)	
High exposure	211 (22.8%)	51 (18.1%)	46 (25.4%)	114 (24.7%)	
Occupational exposure to irritant without coexposure to HMW agents					
Medium exposure	177 (25.1%)	59 (26.6%)	32 (22.4%)	86 (25.4%)	
High exposure	98 (13.9%)	23 (10.4%)	21 (14.7%)	54 (15.9%)	
Profile of sIgE sensitization					<0.01
Few Allergens	532 (57.6%)	56 (19.9%)	109 (60.2%)	367 (79.6%)	
Pollen/animal allergens	176 (19.0%)	72 (25.5%)	47 (26.0%)	57 (12.4%)	
HDM predominant allergens	216 (23.4%)	154 (54.6%)	25 (13.8%)	37 (8.0%)	
Number of sIgE sensitization	2.00 (0.00, 7.25)	8.00 (4.25, 13.00)	2.00 (0.00, 6.00)	0.00 (0.00, 2.00)	<0.01

[1] **Mean (SD); n (%); Median (IQR)**

Cross sectional association between irritant occupational exposure and sIgE sensitization

Multivariate analysis did not show association between OIE and sIgE sensitization for non-asthmatic participants and children-onset asthmatic participants in term of both number and type of sIgE sensitization (Figure 17). However, among participants with adult-onset asthma, OIE tend to be associated with fewer number of sIgE sensitization (aMR (95%CI) for ever, medium and high exposures were 0.73 (0.48-1.08) p=0.11, 0.82 (0.51-1.31), p=0.40, 0.63(0.39-1.02), p=0.06, respectively, p trend= 0.06) (figure 17 panel A, table 26 in supplements). Concerning the type of sensitization, OIE tended to be associated with a reduced risk to be sensitized to both pollen/animals and HDM predominant allergens type of sensitization (figure 17 panel B, table 27 in supplements). Ever exposure to occupation irritant was significatively associated with a reduced risk to exhibit a HDM predominant allergen profile (OR (95%CI) 0.32 (0.12-0.86) p=0.02) with a trend in both medium and high exposures (OR respectively 0.32 (0.09-1.14) p= 0.08, 0.32 (0.09-1.11) p=0.07, p trend= 0.04. Compared to non-exposed adult-onset asthmatic participants, exposure to occupational irritants was associated with a reduced risk to exhibit pollen/animal allergens sensitization profile (OR for ever, medium and high exposure were 0.55 (0.26-1.19), p=0.13, 0.76 (0.31-1.86) p=0.55 and 0.37 (0.13- 1.02), respectively, p trend= 0.06). Sex and smoking did not modify the OIE and sIgE sensitization in the overall population (p interaction >0.2 for all exposures, results not shown). After exclusion of participants co-exposed to HMW agents, similar pattern of association was observed (table 26 and 28 in supplements)

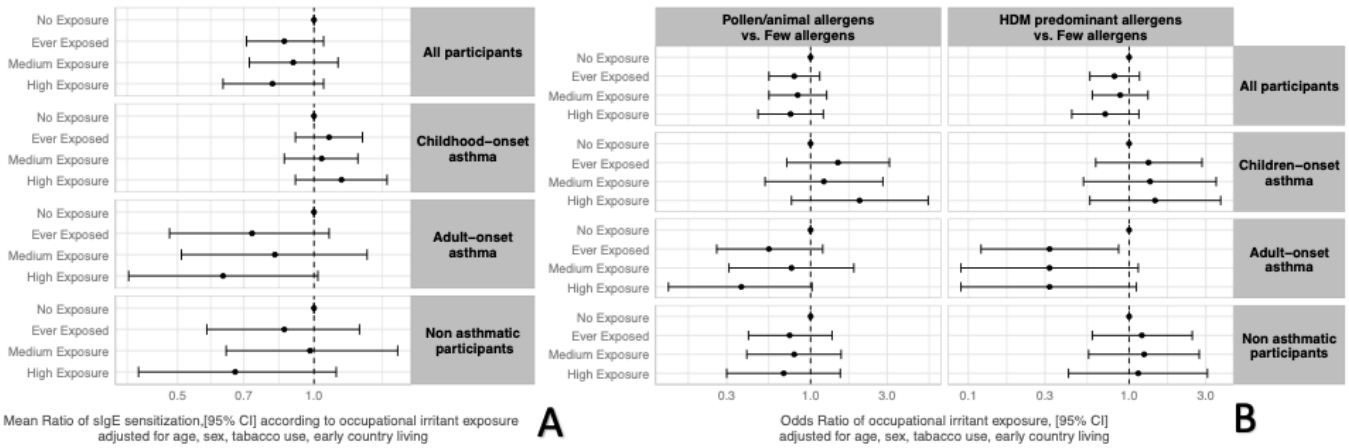

Figure 17 Association of occupational irritant exposure with the number of sIgE sensitization (panel A) and the type of of sIgE sensitization (panel B) according to asthma status at EGEA 2

Longitudinal association between childhood sIgE sensitization and occupation irritant exposures during adulthood

This secondary analysis investigated a potential healthy hire effect among 271 children (mean(sd) age= 11.94 (2.36); 52% with asthma) in EGEA1 followed-up to adulthood in EGEA2 (Table 24). Children with asthma were more frequently male and exhibited a high number of sIgE sensitization as compared to those without asthma. Results from the multivariate regression analysis did not show association between number of sIgE sensitization during childhood and occupational irritant exposure during adulthood (Table 25).

Table 24 Longitudinal associations between sIgE sensitization at EGEA1 and the level of occupational irritant exposure at EGEA 2

Characteristic	Overall, **N = 271[1]**	Non asthmatic subjets **N = 130[1]**	Asthmatic subjets **N = 141[1]**	p value
Age, year	11.94 (2.36)	12.28 (2.39)	11.63 (2.29)	0.01
Sex, female	128 (47.2%)	76 (58.5%)	52 (36.9%)	<0.01
Early country living	9 (3.4%)	6 (4.7%)	3 (2.2%)	0.32
Unknown	5	2	3	
Father diploma level				0.14
Primary	53 (25.2%)	14 (17.7%)	39 (29.8%)	
Secondary	44 (21.0%)	17 (21.5%)	27 (20.6%)	
Post Secondary	113 (53.8%)	48 (60.8%)	65 (49.6%)	
Unknown	*61*	*51*	*10*	
Asthma severity				
mild	-	-	80 (85.1%)	
moderate to severe	-	-	14 (14.9%)	
Unknown	-	-	*47*	
Number of sIgE sensitization	4.00 (0.00, 10.00)	1.00 (0.00, 3.00)	9.00 (5.00, 12.00)	<0.01
Follow up exposure data				
Occupational exposure to irritant at EGEA 2				0.36
Never	141 (52.0%)	62 (47.7%)	79 (56.0%)	
Medium exposure	92 (33.9%)	49 (37.7%)	43 (30.5%)	
High exposure	38 (14.0%)	19 (14.6%)	19 (13.5%)	

[1] Mean (SD); n (%); Median (IQR)

Table 25 Longitudinal associations between sIgE sensitization at EGEA1 and the level of occupational irritant exposure at EGEA 2

		Not exposed (n=141)	Exposed[1] (n=130)	p value	Medium exposure[1] (n= 92)	p value	High exposure[1] (n=38)	p value
Number of sIgE sensitization	**All children(n=271)**	ref	1.01 (0.96-1.05)	0.79	1.01 (0.96-1.05)	0.83	1.01 (0.95-1.07)	0.80
	Children with asthma (n=141)	ref	1.05 (0.99 1.12)	0.11	1.05 (0.98-1.13)	0.13	1.05 (0.96-1.15)	0.28
	Children without asthma (n=130)	ref	1.06 (0.97-1.17)	0.22	1.02 (0.92-1.14)	0.71	1.10 (0.96-1.27)	0.17

1: Odd Ratio (95% Confidence Interval) Adjusted for age and sex and father educational level

8.5 Discussion

To the best of our knowledge, this study is the first to thoroughly examine the association between irritant occupational exposure and allergic sensitization, using allergen-specific measurement to a large number of respiratory allergen molecules. In participants with adult-onset asthma, IOE tended to be associated with fewer IgE-reactive allergens. This association for fewer sIgE sensitization was not specific of a particular profile of allergic sensitization, as a trend for a reduced risk was observed both for HDM and pollen/animal predominant allergen profiles. Our study did not show any association among participants with childhood-onset asthma and those without asthma. The lack of association between sIgE sensitization in childhood with OIE about 12 years later in adulthood suggests no healthy hire effect related to sIgE-reactivity.

Our study, based on a well-characterized allergic sensitization definition, extends results from previous studies indicating that asthma associated with exposure to cleaning agent , Low Molecular Weight (LMW) agents or non-specific irritants, were more likely to be non-atopic [344,346,347,446]. In a study on cleaning workers, participants with a history of asthma were

more often atopic than healthy worker [450]. This result is difficult to interpret as only 17% of population had adult-onset asthma, without asthmatic participant free from occupational exposure.

Several interpretations of the observed negative association between OIE and sIgE sensitization are possible. First, a such negative association could be explained by a healthy hire effect, a specific selection bias related to the phenomenon that due to their health status some individuals may choose work with low occupation exposure [367]. Asthma with onset before completion of full-time education was associated with a lower risk of occupational exposure to dust, gases or fumes [458]. Severe asthma in childhood has also been associated with a decrease risk of being exposed to asthmogens. However, with regard to atopy, results are inconsistent with some studies showing negative [454], no [368,452,458] or positive [453] association between allergic rhinitis in childhood and occupational exposure to asthmogens in adulthood. In addition, using prospective data, sIgE sensitization during childhood was not associated with subsequent irritant occupational exposures in our study, which suggests the absence of healthy hire effect based on sIgE sensitization.

Secondly, the association observed could result from a collider bias. Collider bias occurs when both the exposure (e.g. OIE) and the outcome (e.g. sIgE sentitization) independently cause a common third variable (e.g. collider), that is controlled by design or analysis in the experiment [459]. In the study population, about 50% of the participants exhibited asthma, and both sIgE sensitization [366] and IOE [339] are determinants of asthma. That's why no adjustment was made on asthma status and stratification was preferred, but a collider bias cannot be dismiss among participants with asthma.

Thirdly the association of OIE with sIgE could result from a specific effect of the irritant agent on sIgE sensitization. A number of epidemiological studies have indicated that the level of airborne exposure to specific substances may exert an influence on the atopic status of

individuals [460]. Occupational exposure to endotoxin in adult have been shown to be associated with a reduced prevalence of hay fever and IgE sensitization independently of farming exposure during childhood [461,462]. Interestingly, tobacco smoke has also been described as negatively associated to sIgE sensitization in both cross sectional and longitudinal setting [463,464]. Baur *et al.* described that a longer exposure to isocyanate was associated with a reduced probability of exhibiting IgE sensitization [460]. Zock and coworkers described in a ECRHS cross sectional study that the use of bleach was negatively associated with atopic sensitization and positively associated with non-allergic respiratory symptoms [447]. Nevertheless, in this study, OIE tend to be associated with fewer sensitization only in adult-onset asthma which do not support this hypothesis.

Another interpretation could also be that irritant induced asthma develops predominantly in participants exhibiting a few allergen sIgE sensitization profile. In RHINE study, a large longitudinal population-based study with a ten year follow up, non-atopic participants exposed to low molecular weight agents (LMW) and irritants were more at risk to develop asthma than atopic participants [343]. Dumas *et al.* have clearly proven the association between chronic irritant exposure and asthma in a large Estonian cohort in 2014 [341]. This association had not been consistently found in previous European studies using the same job exposure matrix [341,465,466]. A hypothesis was then put forward to explain the discrepancy in results. It has been suggested that the Estonian population, having been born and raised in a Soviet environment, were more likely to be free from IgE sensitization and thus more susceptible to developing irritant-induced asthma. [341]. Non sensitized individuals might be more vulnerable to OIE. In ECRHS II, le Moual and coworkers described an association between uncontrolled adult-onset asthma and occupational exposure to cleaning agents and LMW agents [467]. The association was higher in adult-onset asthmatic non sensitized to

common aeroallergens compared to adult-onset participants who exhibited at least one IgE sensitization.

Mechanisms of irritant induced asthma are not clearly elucidated. Sensory irritation and tissue irritation are the two main mechanisms proposed [339]. Recently, using unsupervised cluster classification, IOE have been associated with a cluster gathering mostly adult-onset asthma, with poor lung function, high blood neutrophils counts and high fluorescent oxidation products [345]. Interestingly, this cluster exhibited the highest rate of participants without IgE sensitization among asthmatic patients [468], supporting the hypothesis that irritant induced asthma may develop through non immunologic mechanisms. Only scarce information is available about phenotypic traits of irritant induced asthma as for most of cases the level of evidence of the causal relationship between workplace exposures and the development of asthma is low [331]. Lantto et al. [338] described asthma outcome for a subset of definite and probable irritant induced asthma six months after the diagnostic and observed a lower proportion of atopic subject in irritant induced asthma than in HMW sensitized subjects.

One strength of this study is the quantification of sIgE sensitization by micro array technology which enable a quantitative and qualitative evaluation of sIgE sensitization. This approach avoids issues related to multiple testing and account for potential additive or multiplicative effects of IgE-reactivity to allergen components. Another strength is the assessment method of IOE, made via the OAsJEM. Job exposure matrices have been shown to reduce the risk misclassification bias between asthmatic and non-asthmatic in comparison with self-report surveys [359]. The OAsJEM allows evaluation of retrospective occupational exposures assessment to 30 specific asthmagens and irritants for all ISCO-88 job codes, it has been shown efficient to prove association between occupational exposure and asthma in a recent large population based study [342].

Our study has also limitations. Although the study is relatively large, one significant limitation is the lack of statistical power. Stratifying by asthma status also results in a reduction in the size of the population and a limited number of participants in some exposure groups, which in turn leads to wide confidence intervals. We were also not able to study independently LMW sensitize and irritant agent because of the lack of statistical power. In the current study, the chronology of asthma onset with IOE was not ascertain, which prevent strong conclusions on the effect of irritants on adult-onset asthma. The results of this study indicate a need for further investigation in a longitudinal setting to elucidate whether IOE induce lower sensitization or elicit asthma in individuals with few allergens sensitization profile.

In conclusion, in the EGEA population, irritant occupational exposures tended to be associated with fewer sIgE sensitization in adult-onset asthma irrespectively of the type of sensitization. Despite its inherent limitations, our findings contribute to provide new insight into the understanding of asthma and its heterogeneity. The elucidation of mechanisms by which irritants induce asthma may provide a clue to the link between irritant occupational exposures and sIgE sensitization.

8.6 Supplements

Table 26 Cross-sectional associations of the number of sIgE sensitization at EGEA 2 with the level of occupational irritant exposure

	Number of sIgE sensitization								
	In all patients					After exclusion of patients co-exposed to HMW			
	n	%	aMR (95% CI) [1]	p value	p trend	n	%	aMR (95% CI) [1]	p value	p trend
All	924					704				
No exposure	429	46.4	Ref			429	61	Ref		
Exposed	495	53.6	0.86 (0.71-1.05)	0.14		279	39	0.83 (0.66-1.04)	0.10	
Medium exposure	284	30.7	0.90 (0.72-1.13)	0.36	0.10	177	25.1	0.89 (0.69-1.14)	0.35	**0.043**
High exposure	211	22.8	0.81 (0.63-1.05)	0.11		98	13.9	0.71 (0.50-0.99)	**0.044**	
Childhood-onset Asthma	282					222				
No exposure	140	49.6	Ref			140	63	Ref		
Exposed	142	50.4	1.08 (0.91-1.28)	0.40		82	37	1.02 (0.84-1.25)	0.82	
Medium exposure	91	32.3	1.04 (0.86-1.25)	0.72	0.27	59	26.6	1.05 (0.84-1.31)	0.68	0.99
High exposure	51	18.1	1.15 (0.91-1.45)	0.24		23	10.4	0.95 (0.69-1.33)	0.78	
Adult-onset Asthma	181					143				
No exposure	90	49.7	Ref			90	62.9	Ref		
Exposed	91	50.3	0.73 (0.48-1.08)	0.11		53	37.1	0.76 (0.48-1.22)	0.25	
Medium exposure	45	24.9	0.82 (0.51-1.31)	0.40	0.060	32	22.4	0.87 (0.52-1.46)	0.59	0.095
High exposure	46	25.4	0.63 (0.39-1.02)	0.061		21	14.7	0.53 (0.27-1.06)	0.072	
Non asthmatic participants	461					339				
No exposure	199	43.2	Ref			199	58.7	Ref		
Exposed	262	56.8	0.86 (0.58-1.26)	0.44		140	41.3	0.81 (0.51-1.28)	0.37	
Medium exposure	148	32.1	0.98 (0.64-1.53)	0.94	0.17	86	25.4	0.79 (0.47-1.34)	0.38	0.46
High exposure	114	24.7	0.67 (0.41-1.12)	0.13		54	15.9	0.84 (0.43-1.63)	0.60	

1: Mean ratio of sIgE sensitization adjusted for age, sex, tobacco use and early country living

Table 27 Cross-sectional association of the level of irritant occupational exposure with sIgE sensitization profile at EGEA 2

	n	% exposed	Adjusted OR (95% CI) * Vs. Few Allergens (n=532)					
			Pollen/animal allergens (n=176)	p value	p trend	HDM predominant allergens (n=216)	p value	p trend
All	924							
No exposure	429	46.4	Ref			Ref		
Exposed	495	53.6	0.79 (0.55-1.14)	0.21		0.81 (0.57-1.16)	0.25	
Medium exposure	284	30.7	0.83 (0.55-1.26)	0.37	0.20	0.88 (0.59-1.31)	0.53	0.17
High exposure	211	22.8	0.75 (0.47-1.20)	0.23		0.71 (0.44-1.15)	0.16	
Childhood-onset Asthma	282							
No exposure	140	49.6	Ref			Ref		
Exposed	142	50.4	1.48 (0.71-3.10)	0.29		1.32 (0.62-2.84)	0.47	
Medium exposure	91	32.3	1.21 (0.52-2.82)	0.66	0.17	1.35 (0.52-3.48)	0.53	0.44
High exposure	51	18.1	2.02 (0.76-5.40)	0.16		1.45 (0.57-3.71)	0.43	
Adult-onset Asthma	181							
No exposure	90	49.7	Ref			Ref		
Exposed	91	50.3	0.55 (0.26-1.19)	0.13		0.32 (0.12-0.86)	**0.024**	
Medium exposure	45	24.9	0.76 (0.31-1.86)	0.55	0.063	0.32 (0.09-1.14)	0.080	0.041
High exposure	46	25.4	0.37 (0.13-1.02)	0.055		0.32 (0.09-1.11)	0.072	
Non asthmatic participants	461							
No exposure	199	43.2	Ref			Ref		
Exposed	262	56.8	0.74 (0.41-1.36)	0.34	0.32	1.20 (0.59-2.47)	0.61	0.71
Medium exposure	148	32.1	0.79 (0.40-1.55)	0.49		1.24 (0.56-2.73)	0.59	
High exposure	114	24.7	0.68 (0.30-1.53)	0.35		1.14 (0.42-3.07)	0.80	

*** adjusted for age, sex, tobacco use and early country living;**

Table 28 Cross-sectional association of the level of irritant occupational exposure with sIgE sensitization profile at EGEA 2 in participants without co exposure to HMW agents

	n	%	Adjusted OR (95% CI)* Vs. Few Allergens (n=399)					
			Pollen/animal allergens (n=135)	p value	p trend	HDM predominant allergens (n=170)	p value	p trend
All	704							
No exposure	429	61	Ref			Ref		
Exposed	279	39	0.71 (0.46-1.10)	0.13		0.73 (0.48-1.12)	0.15	
Medium exposure	177	25.1	0.84 (0.52-1.36)	0.49	0.049	0.78 (0.48-1.25)	0.30	0.12
High exposure	98	13.9	0.48 (0.24-0.97)	**0.040**		0.64 (0.33-1.23)	0.18	
Childhood-onset Asthma	222							
No exposure	140	63	Ref			Ref		
Exposed	82	37	1.29 (0.54-3.12)	0.56		1.06 (0.48-2.33)	0.89	
Medium exposure	59	26.6	1.26 (0.48-3.36)	0.64	0.77	1.03 (0.43-2.49)	0.95	0.87
High exposure	23	10.4	1.37 (0.34-5.48)	0.65		1.12 (0.32-3.95)	0.86	
Adult-onset Asthma	143							
No exposure	90	62.9	Ref			Ref		
Exposed	53	37.1	0.54 (0.20-1.41)	0.20		0.39 (0.12-1.29)	0.12	
Medium exposure	32	22.4	0.86 (0.31-2.43)	0.76	0.062	0.41 (0.10-1.73)	0.22	0.062
High exposure	21	14.7	0.11 (0.01-1.00)	**0.050**		0.38 (0.07-2.12)	0.27	
Non asthmatic participants	339							
No exposure	199	58.7	Ref			Ref		
Exposed	140	41.3	0.65 (0.32-1.34)	0.24		1.01 (0.42-2.41)	0.99	
Medium exposure	86	25.4	0.69 (0.30-1.58)	0.38	0.25	0.85 (0.31-2.37)	0.76	0.75
High exposure	54	15.9	0.59 (0.21-1.66)	0.31		1.44 (0.40-5.11)	0.58	

***adjusted for age, sex, tobacco use and early country living**

Part 9: General discussion and perspectives

9.1 Summary of the main findings and interpretation:

Occupational asthma has been described as being a specific phenotype of asthma [21]. The aim of this research was to study the heterogeneity of occupational asthma in order to define specific OA phenotypes, with the ultimate goal of providing essential information to improve the management of occupational asthma. A multidimensional approach was followed, based on a cohort of clinically validated cases with occupational asthma and on an epidemiological cohort enriched with participants with asthma, and by addressing the research question by focusing on different component: the offending agent, the clinical and functional presentation, and the inflammatory patterns. Each of these components seems to explain partly the heterogeneity of the disease. Each of these characterizations seems to distinguish groups of patients, yet there is also various degree of overlap between them. Indeed, several questions remain.

How to classify sensitizer-induced asthma?

We could expect of a classification to distinguish individuals in terms of disease mechanism. In this thesis work, we have shown that both HMW and LMW-induced asthma exhibited predominantly eosinophilic inflammation. One question would be if it is preferable to characterize sensitizer-induced OA by its inflammatory pattern or by its molecular weight. The situation could be compared to the controversy in the 90's about intrinsic and extrinsic asthma. Bronchial biopsy studies had found similar immunopathological entity between atopic and non-atopic asthma [34]. It was suggested that, despite different clinical profile, intrinsic asthma could also be IgE mediated, by unknown antigen, or allergen with local IgE production [36]. Nowadays, allergic asthma is still considered as distinct phenotype, even by some as an

endotype, because of specific pathobiological pathway and specific management such as environmental control measures and allergen specific immunotherapy [50]. Non atopic asthma has been disentangled in multiple phenotypes, eosinophilic inflammation in non-atopic subjects has been explained by the ILC2 pathway. I support that HMW induced, or more precisely, IgE-mediated OA is a distinct phenotype of sensitizer-induced asthma because it gathers common clinical characteristic, risk factors and potential treatments. Development of standardized extracts and development of studies to assess the efficacy of AIT in IgE mediated OA are to be supported [319].

Assuming that HMW and LMW induced asthma have the same underlying mechanism because both predominantly eosinophilic would be in my point of view a false syllogism. One notable example of a false syllogism can be observed in the opening act of Ionesco's play, Rhinoceros:" All cats die. Socrates is dead. Therefore, Socrates is a cat" [469]. Discrepancy between T2 biomarkers between HMW and LMW induced asthma may suggest different molecular pathways for each type of OA leading both to an eosinophilic inflammation. Compared to LMW induced asthma, HMW induced asthma has been associated with an increase of FeNO during SIC [280,330]. For a long time FeNO measurement have been seen as a simple surrogate marker for induced sputum in order to assess T2 inflammation [223]. Recent findings support the idea the FeNO is rather a parallel marker of airway inflammation [470,471]. Indeed, inducible nitric oxide synthase (iNOS) is activated by STAT6, itself upregulated by IL-4 and IL-13. However, IL-5 upregulates STAT5 which does not have an effect on iNOS or FENO levels [470,471]. This differential pathway between FeNO and sputum eosinophils explains why 1) baseline FeNO level is not predictive of a response to an anti IL-5 or anti IL-5R therapy in severe asthmatic patients 2) no change of FeNO is observe in severe asthmatic patients treated by anti IL-5 or anti IL-5R therapy [472,473]. It could be hypothesized that LMW elicit an IL-5 predominant inflammation, which could explain an increase of eosinophils without a significant

change of FeNO. This hypothesis would be in accordance with the findings of Jones and coworkers who constated a striking absence of C ε and IL-4 positive cells in bronchial biopsy specimens from patients challenged with diisocyanate [474]. On the other hand, they found an increased numbers of IL-5, CD25 and CD4 positive cells. One other study of diisocyanate-induced asthma demonstrated that most T cells on bronchial biopsy exhibited the CD8 phenotype and produced IFN-gamma and IL-5, with few T cells producing the Th2 cytokine IL-4 [475]. IL 5 have been described in non-occupational asthma to be mainly involved in the later phase of the immune response where eosinophils play a more active role [476,477]. Interestingly, LMW induced asthma has been found to be associated with late asthmatic reaction during SIC compared to HMW induced asthma [280].

Recently, the presence of ILC2 cells have been demonstrated in human biopsies of subjects with diisocyanate induced asthma [478]. ILC2s and Th2 cells have identical functions and similar requirement of transcriptional machinery in cytokine production. Yet, due to the lack of an antigen receptor and toll-like receptor, ILC2s mainly respond to cytokines such as IL-33 to produce type 2 cytokines, IL-5 and IL-13, but not IL-4 [479]. The predominance of IL-5 inflammation in LMW induced asthma could be explained by an ILC2 prone inflammation.

Type V hypersensitivity has been developed in the new nomenclature of allergic diseases [39]. It concerns hypersensitivity reactions associated with an epithelial barrier defect caused by environmental factors which result in dysregulation of the immune response. It has been demonstrated in mouse that intranasal administration of commercially available laundry detergents can induce eosinophilic airway inflammation in vivo through ILC2 activation [480]. They have shown that the T2 inflammatory response was initiated by increased expression of IL-33 in the airway epithelial cells due to oxidative stress. Also, this reaction could occur without the involvement of adaptative immunity. Whether LMW agent could elicit type V hypersensitivity remains to be investigated.

Can we still classify OA only in term of offending agent?

It can be reasonably deduced from the extant literature that LMW-induced asthma constitutes a heterogenous group. As presented in the introduction, it has been described “atypical LMW agents” that elicit IgE mediated reactions such as acid anhydride, chloramine T, platinum salts. Here we observed that QAC induced OA was independently associated with a higher eosinophilic response. Moreover, compared to other LMW induced asthma, QAC-induced asthmatic patients did not exhibit phenotypic characteristic associated to HMW induced asthma (eg. higher frequence of work-related rhinitis or increase of FeNO during SIC).

In addition, we have seen that a sensitizing occupational agent can induce OA through different inflammatory pathways, independently of its HMW or LMW category. Attempting to apply a diagnostic label to people with overlapping phenotypes can cause confusion for clinicians [265]. If one refers to an inflammatory phenotype classification, allergic asthma belongs to the 'eosinophilic' phenotype. We have observed subset of patients sensitized to HMW agents exhibiting neutrophilic inflammation. A measure bias resulting to induced sputum technique cannot be excluded. Nevertheless, this constatation have also been drawn in non-occupational allergic asthma [438]. Moreover, neutrophils have been shown to have a potential pro-inflammatory role in the IgE mediated reaction which warrant further investigations [435].

There is increasing evidence that most LMW agent can induce asthma by both sensitizing or irritant mechanisms [481,482]. It has been suggested, for some chemicals (eg. isocyanate, anhydrides, formaldehyde, or some disinfectant) , that sensitizer and irritant properties might depend on the level/intensity of exposure [331,481]. It is my belief that host factors may also be important in the development of both mechanisms. The work performed in the EGEA study suggests that individuals that exhibit few sIgE sensitization profile might be more vulnerable to irritant products. LMW-induced asthma have been associated to genetic susceptibility,

mainly HLA genes [286]. It could therefore be hypothesized that irritant induced asthma could also be associated with other genetic susceptibilities. Indeed, gene-environment interaction are sought to be important to understand the heritability of asthma [483]. The treatable trait framework may help to avoid misclassification of patients as it does not assume a molecular pathway from any *a priori* classification.

Does the distinction between occupational or non-occupational asthma enable to distinguish different molecular pathway?

As previously said, we could expect of a classification to distinguish individuals in terms of disease mechanism. In non-occupational asthma, many efforts have been made to look for endotypes of asthma. Whether occupational asthma result from distinct endotypes is a subject of interest and has been one of the main motivations of the creation of the E-PHOCAS cohort [345,484,485]. I do not support the hypothesis that such "occupational endotypes" exist, as IgE-mediated inflammation nor irritant induced mechanisms are specific of occupational settings. Indeed, IgE mediated asthma is the most common asthma endotype, and non-occupational exposure to irritants (eg. cleaning products) have been shown to be associated with both asthma incidence and loss of control of asthma independently to sensitizing mechanisms [486,487]. Most LMW induced asthma result from an uncharacterized type of allergy. Nevertheless, nothing guarantee that its mechanism is specific of occupational setting, the nomenclature of allergic diseases is still evolving [39].

Why it is crucial to talk about occupational asthma and work-related asthma?

The main reason is because asthmatic patients' heterogeneity cannot be reduced to a molecular pathway or a response's treatment. The current therapeutic approach, based on the treatment of the inflammation, have led to a decrease of asthma related death in the 80's until the mid-2000s [262,488]. The development of biologic therapies in asthma have enable to make decrease the

disease burden of severe asthma [241]. However, asthma-related mortality has been stagnating for several years [488–491]. Guilleminault and coworkers conducted a descriptive study of patients who died from asthma between 2013 and 2017 using data from the French National Health Data System [492]. Half of the patients who died from asthma received inadequate ICS doses and less than 15% were referred to a specialist. Health disparities in asthma continue despite the presence of safe and effective treatment [493–495]. Poor compliance and poor access to the healthcare system are key determinants of asthma outcomes.

Work is a significant contributor to health disparities, affecting financial status, health care access, and exposure to hazardous substances [496]. Population-based surveys have shown that the type of work more at risk for work-related asthma are often low-paying jobs [497]. Additionally, research has indicated a higher prevalence of work-related asthma among ethnic minorities and individuals with lower levels of education compared to white people or those with more education [496,498]. Work-related asthma patients are more likely to become unemployed and the rate of both unemployment or job change have been found to be similar in OA and in WEA [275]. It is not uncommon for workers with work related asthma to refrain from reporting asthma symptoms or exposures in the workplace. This may be due to a number of factors, including a perceived lack of solutions, concerns about job security, apprehension about confirming a diagnosis, limited awareness of potential work-related asthma triggers or associations between symptoms and occupational factors, and inadequate access to healthcare resources.

Work-related asthma patients has been described to exhibit worse asthma outcomes than non-work-related asthma individuals in term of both control and emergency care visit [248,499,500]. Sensitizer-induced asthma has been estimated to be severe in more than 16% of cases [299]. Main determinants of severe sensitizer induced asthma were the persistence of exposure, the duration of the disease and low level of education. One other additional

explanation could be the presence of comorbidity associated with work-related asthma. There is emerging evidence that asthma comorbidities may be related with asthma phenotypes and its evolution. An analysis of the Tasmanian Longitudinal Health Study showed that distinct longitudinal trajectories of asthma and allergic disease from 7 to 53 years of age were associated with different profiles of extrapulmonary comorbidities [501]. In this thesis, was shown that work related dysphonia, was prevalent in our population of sensitizer induced asthma. Patients experiencing work related dysphonia tended to exhibit high level of treatment. It may be suggested that these patients might be over treated because of the coexistence of the comorbidity as they also exhibited lower eosinophilic sputum count. Additionally, other comorbidities have been shown to be associated with both OA and WEA. Compared to non-asthmatic workers, OA and WEA individuals exhibit a higher risk of anxiety, psychiatric disorder and impaired quality of life [502,503].

Work related asthma could be entirely preventable through elimination of exposure to causal agents in the workplace. Even though elimination of exposure is not always possible, control measures of exposition can be implemented and substitution of alternative agents can be a solution. The substitution of powdered latex gloves with low-protein non-powder gloves has been demonstrated to be an effective primary prevention measure for the elimination of natural rubber latex allergen sensitization, contact urticaria and occupational asthma among healthcare workers [504,505]. As work related asthma may represent one about four asthmatic patients, the impact of work on asthma symptoms should be assessed systematically, which is not actually performed [271]. Pulmonologist and allergologist should incorporate an occupational history as part of the routine evaluation of all working patients with asthma and collaboration with occupational health physician should be enticed.

9.2 Strength and limitations

One main strength of the E-PHOCAS cohort is the multicentric design and the method used to diagnose sensitizer induced asthma. The SIC results were interpreted a posteriori according to standardized and homogeneous criteria following consensus statement of the European Respiratory Society [284]. Reproducing asthma reaction using realistic method has enables to establish the causality of QAC exposure to sensitizer induce asthma.

Moreover, the E-PHOCAS cohort gathered a large range of different occupational agents. To my knowledge the E-PHOCAS cohort is the largest cohort of patients on sensitizer induced asthma. This is all the more important as the number of subjects is often a limitation in occupational asthma studies. Additionally, inflammatory profiles were established via induced sputum technique that has been proven to be safe, reproducible and be an accurate surrogate to invasive techniques [218–220].

Nevertheless, this cohort has limitations. First, the data pertaining respiratory symptoms were not collected through a standardized questionnaire. Validated questionnaire on asthma control would have been more appropriate. Also, the sputum induction technique and processing were not similar in all centers. Indeed, variability of both neutrophil and eosinophil counts have been observed through the different centers. Finally, the retrospective design did not allow to evaluate the coherence of phenotypes in time and to define trajectories.

Concerning EGEA study, one strength is the assessment of sIgE via micro array technology in a well-defined population using latent class analysis to identify profile of sIgE sensitization. Also, occupational exposures were estimated via recently updated job exposure matrix specific of occupational asthma. In addition, available data in childhood participants enable to exclude a healthy hire effect. Nevertheless, the study is limited by the number of participants which was low after stratification by asthma status, resulting in a lack of power. The same

lack of power did not allow the evaluation of LMW sensitizer independently to irritant occupational agents. Also, the negative association observed between IOE and sIgE sensitization in adult-onset asthma could result from a collider bias which could not be avoided in this population even after stratification on asthma status. To finish, the cross-sectional setting precludes interpretation of the direction of the association between IOE and sIgE sensitization in adult onset asthmatic participants.

9.3 Perspectives

Whether eosinophilic inflammation in sensitizer-induced OA is predictive of worse long-term outcome remains unknown. Two follow up studies in OA have been performed, showing contradictory results [247,251]. Lemiere *et al* suggested that an eosinophilic response during specific inhalation challenge was a predictive factor of a good evolution in terms of inhaled corticosteroid use and airflow obstruction, when comparing the evolution of 36 patients 5 years after diagnosis [247]. Conversely, Talini *et al.* described the evolution of 38 participants over 5 years in which eosinophilic inflammation at baseline was a determinant of FEV1 decline [251] . On the other hand, elevated T2 biomarker levels have been associated with a decline in lung function both in healthy individuals and in patients with non-occupational asthma [25,506–508]. A prospective study through the E-PHOCAS cohort could provide insights in the relationship between T2 inflammation and long-term outcome in order to enhance precision of the management of patient at diagnostic. In order to do so, a standardisation of data collection and technique of sputum induction is needed because heterogeneity of characterisation of inflammatory pattern in the different centers have been observed.

A precise characterisation of both extra pulmonary and behavioural/risk factor traits could be performed via the E-PHOCAS network. Even though comorbidities such as depression and anxiety have been already assessed, a precise characterisation of dysfunctional breathing, sarcopenia and ILO could guide non-pharmacologic management of sensitizer-induced asthma. The determination of health disparities according to geographical centrers could provide insights into the impact of different healthcare systems and the differential recognition of occupational disease across countries on asthma outcomes. In order to ascertain the social impact of WEA, which may not differ from that of OA, it would be beneficial to include WEA patients in the E-PHOCAS network. This would help to highlight the need for recognition from the healthcare system.

Facilitate more efficient diagnosis of work-related asthma is an urgent unmet need. Indeed, SIC is available in only limited number of institutions which cannot guarantee an equal access to all. Studying variation of FeNO or induced sputum eosinophil count at work and off work might be useful to identify sensitizer induced asthma. Additionally, mobile health has the potential to transform the face of heath service delivery across the globe according to the World Heath Organisation [509,510]. Mobile Health tools for work related asthma diagnosis could be developed. For example, digital peak flow meter have been found to be able to increase patients' adherence and to monitor important asthma outcomes such as daily symptoms' severity, reliever medication usage and the risk for asthma worsening via an Artificial Intelligent algorithm [511].

Concerning irritant induced asthma, longitudinal study on incidence of irritant induced asthma is needed to increase our understanding of its mechanisms and its determinants. Investigating gene by environment interaction and epigenetic mechanisms might help to identify modulating factors. Longitudinal study could also provide population attributable fraction specifically for occupational irritants exposures which is lacking, and thus, contribute to alert authorities of the burden that they represent.

Part 10: General conclusion

This PhD work aimed to provide better insights in occupational asthma phenotypes. It has been shown that key elements of the characterization of non-occupational asthma such as inflammatory pattern, comorbidities also distinguish phenotypes in occupational asthma. Moreover, was observed that a sensitizing occupational agent can induce OA through different inflammatory pathways, independently of its HMW or LMW category. These results challenge the historical classification of occupational asthma which relies exclusively on the type of the offending agent. It supports, according to me, the treatable trait framework that state that heterogeneity should be handle at individual level to provide precise medicine. In my view, occupational asthma is not a specific phenotype of asthma. It is driven by mechanisms that are also present in non-occupational setting such as IgE mediated asthma and irritant-induced asthma. Indeed, irritant-induced mechanism has been evoked to be elicited by non-occupational exposures such as household cleaning products. Nevertheless, work-related asthma should be considered as a major “lifestyle/risk factor” treatable trait because associated with a high socio-economic burden and also with comorbidities such as anxiety and depression that are to be considered in a multi-disciplinary way in the management.

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Résumé détaillé :

1.Introduction et contexte du travail :

L'asthme est une maladie respiratoire chronique fréquente qui touche 5.8 % de la population adulte française (5.1 % des hommes, 6.4 % des femmes). La pathologie asthmatique est un enjeu de santé publique car elle est associée à un lourd fardeau tant en termes de prévalence qu'en terme d'espérance de vie en bonne santé et en termes de coût de santé.

Malgré des critères diagnostiques bien établis, l'asthme comporte des présentations cliniques diverses et des réponses aux traitements variables, faisant suspecter des mécanismes physiopathologiques sous-jacents distincts. Les dernières décennies ont permis de voir émerger une caractérisation plus fine de la maladie, via un phénotypage selon les facteurs déclencheurs, les formes cliniques, et les mécanismes inflammatoires, qui ont permis de s'approcher d'une médecine de précision.

L'asthme professionnel, défini comme un asthme issu d'une exposition professionnelle et ne pouvant être attribué à des causes extérieures au lieu de travail, représente 16 % des cas d'asthme. Sa classification repose essentiellement sur des constatations empiriques mises en évidence dans les années 80. L'asthme professionnel, était considéré comme un modèle pouvant permettre d'accroitre nos connaissances concernant les mécanismes de l'asthme non professionnel car survenant chez des sujets préalablement sains avant l'exposition et dont l'éviction de l'exposition causale pouvait être réalisée après la survenue de la maladie. L'hypothèse de ce travail de thèse est que les avancées réalisées dans la compréhension de l'hétérogénéité de l'asthme non professionnel pourraient permettre une meilleure caractérisation des patients atteints d'asthme professionnel.

La première partie de ce travail résidera en un état des lieux du phénotypage de l'asthme non professionnel, puis un rappel sur la classification actuelle de l'asthme professionnel avant la présentation des méthodes et des résultats.

2. Phénotypage de l'asthme non professionnel

2.1 Phénotypage de l'asthme par type de déclencheur :

L'asthme allergique est le phénotype le plus fréquemment retrouvé. Il représente 80% des asthmes de l'enfant et chez l'adulte, 40 à 50% des asthmes sont considérés comme étant des asthmes allergiques. Ils découlent d'une réaction d'hypersensibilité de type 1 à la suite d'un contact avec un allergène. Cette réaction est constituée de deux phases. La première phase est une phase de sensibilisation qui va voir se développer, via des cellules de l'inflammation de type 2, la production d'IgE spécifiques de l'allergène à la suite de l'exposition. La phase effectrice consiste en la libération de médiateurs inflammatoires provenant de la dégranulation de mastocytes activés par le contact avec les IgE ayant reconnu l'allergène à la suite d'une nouvelle exposition. Ce type d'asthme détermine une présentation clinique particulière, tant sur le plan de l'âge d'apparition qui est préférentiellement durant l'enfance que sur la présentation clinique. En effet les asthmes allergiques sont plus fréquemment associés à des comorbidités allergiques comme la rhinite allergique la conjonctivite et la dermatite atopique. Aussi, il existe des traitements spécifiques pour ce type d'asthme comme l'éviction des allergène, l'immunothérapie allergénique ou des biothérapies conçues expressément pour ce type d'asthme (Omalizumab).

Une réaction asthmatique peut survenir chez certains patients après ingestion d'anti inflammatoires non stéroïdiens (AINS). Il caractérisé cliniquement par la présence d'une polypose naso-sinusienne et parfois par une intolérance à l'alcool. Cet asthme en lien avec la prise d'AINS présente une prévalence estimée de 0,3 à 0,9 % en population générale mais

représente jusqu'à 7% de l'ensemble des asthmes et 14% des asthmes sévères. Il dépend d'un mécanisme physiopathologique spécifique résultant d'un déséquilibre de la voie métabolique de la cyclooxygénase provoquant une accumulation de leucotriènes qui va induire une activation de l'inflammation de type 2. Ce type d'asthme se distingue par sa sévérité, avec une probabilité plus élevée d'avoir recours à des traitements par corticoïdes oraux, ainsi qu'une prédisposition à présenter des exacerbations et à développer un trouble ventilatoire obstructif. Des traitements spécifiques existent là aussi, avec des procédures de désensibilisation spécifique qui permettent d'améliorer le control de la maladie asthmatique et de réduire le recours au traitement chirurgical concernant la prise en charge des polypes.

La bronchoconstriction induite par l'effort physique procède d'une obstruction des voies respiratoires à la suite d'un effort physique important survenant chez des sujets ne présentant pas d'asthme dans d'autres circonstances. Le mécanisme physiopathologique n'est pas déterminé avec certitude mais il est évoqué une réponse cellulaire inflammatoire en lien avec le différentiel de température survenant lors de l'activité physique pendant laquelle le débit d'air est augmenté. La prévalence de cette affection est estimée à 20 % chez l'enfant et l'adulte. Les sports en milieux aquatiques sont plus à risque avec une prévalence allant jusqu'à 40% chez les athlètes de haut niveau. De même, une prise en charge spécifique existe, l'échauffement avant l'effort permettant de réduire la probabilité de survenu de bronchoconstriction.

Nous avons pu constater que la caractérisation de l'asthme en fonction de facteur déclencheur permettait de distinguer les patients asthmatiques en fonction de formes cliniques, de mécanismes, et de réponses au traitement distinct. L'hypothèse peut être faite que de façon similaire, la caractérisation de l'asthme professionnel en fonction du type d'agent puisse permettre de distinguer différents phénotypes d'asthme.

2.2 Phénotypage en fonction de la présentation clinique

L'obésité présente une prévalence estimée à 15% de la population en France en 2016. Nous vivons actuellement une épidémie d'obésité avec une augmentation d'un facteur 4 de la prévalence de l'obésité chez les enfants entre 1990 et 2022. Il a été prouvé via de large méta-analyses que l'incidence d'asthme est associée avec la présence d'une obésité avec une relation dose-réponse. Plusieurs mécanismes sont avancés pour expliquer ce phénomène. Tout d'abord l'accumulation de graisse dans la paroi thoracique a des effets directs sur la mécanique ventilatoire, de plus des facteurs génétiques associés à l'asthme de l'obèse ont été identifiés. Il existe un rôle de l'alimentation qui est de plus en plus étudié notamment concernant l'effet du microbiote sur la survenue d'affections respiratoires. Pour finir il existe des effets métaboliques avec notamment la sécrétion de leptine qui a été montrée comme associée avec l'hyperréactivité bronchique non spécifique. Concernant sa présentation clinique, l'asthme de l'obèse a été décrit comme étant plus sévère, associé au genre féminin et d'apparition plus tardive. Il est établi que l'obésité est un facteur causal de l'asthme chez certains patients, mais il est aussi admis que l'obésité peut aggraver un asthme préexistant. Des interventions spécifiques existent. La perte de poids, soit via l'activité physique, la prise en charge diététique et la chirurgie bariatrique ont une efficacité prouvée pour ce type d'asthme.

La présentation clinique peut etre modifiée du fait de comorbidités associées à l'asthme. L'obstruction laryngée induite, décrit un rétrécissement inapproprié et transitoire du larynx associé à l'exposition à un facteur déclenchant. On estime que cette affection touche jusqu'à 25% de la population asthmatique. Elle appartient à un syndrome plus large que l'on appelle la dysfonction laryngée qui peut allier la dysphonie dysfonctionnelle et la toux chronique réfractaire inexpliquée. Les mécanismes sous-jacents ne sont pas entièrement élucidés. Une dysfonction neurologique des voies respiratoires est suggérée. Ces comorbidités sont surreprésentées chez les sujets asthmatiques. Elles peuvent à la fois mimer l'asthme ou

l'aggraver, induisant un surtraitement de l'asthme si non identifiées. Leur présence est plus fréquente chez les patients asthmatiques présentant une fonction respiratoire préservée, une respiratoire dysfonctionnelle associée et un taux d'éosinophile plus faible. Des traitements spécifiques existent telles la prise en charge orthophonique qui permet d'améliorer la qualité de vie et de diminuer le recours au soin.

De ce fait nous venons d'illustrer que la présentation clinique d'un patient asthmatique peut être révélateur 1) d'un facteur causal (ex : obésité) 2) de comorbidités pouvant être essentielles à identifier afin de réaliser une prise en charge optimale de la pathologie asthmatique.

2.3 Phénotypage de l'asthme selon le type d'inflammation

L'inflammation bronchique est un élément primordial dans la définition de l'asthme. Deux types d'inflammation ont été identifiés. L'inflammation T2 élevée et l'inflammation T2 basse. L'inflammation T2 élevée regroupe l'asthme allergique décrit précédemment, et l'asthme éosinophilique, secondaire à l'exposition aux polluants, virus ou bactéries, provoquant ainsi la libération d'alarmines par l'épithélium respiratoire à l'origine d'une activation de lymphocytes innés de type 2 qui vont activer à leur tour toute la cascade de signalisation de l'inflammation de type 2. L'inflammation T2 basse regroupe l'inflammation neutrophilique, médiée par l'inflammation de type 1 et de type 17 ainsi que les asthmes paucigranulocytiques pour lesquels il n'est pas retrouvé de cellules inflammatoires dans les voies aériennes. Les techniques initiales d'évaluation de l'inflammation bronchique étaient invasives, regroupant la lavage bronchiolo-alvéolaire et les biopsies bronchiques. Par la suite, ont été développées des méthodes non invasives, dont l'expectoration induite qui a fait la preuve d'être un bon estimateur de l'inflammation bronchique. De nombreuses études ont permis de faire le lien entre des

caractéristiques cliniques et fonctionnelles de l'asthme et le type d'inflammation bronchique. L'asthme éosinophilique, correspondant à environ 50% des asthmes est associé à un plus haut niveau d'hyperréactivité bronchique non spécifique, à une fonction respiratoire dégradée et une maladie non contrôlée. L'inflammation neutrophilique, représentant environ 20% des asthmes, était associée à un âge plus avancé, à la présence synchrone d'une obésité, au genre féminin à une moins bonne réponse à la corticothérapie et à une maladie plus sévère. Les asthmes paucigranulocytiques sont décrits comme moins sévères que ce soit en termes de symptômes ou sur le plan fonctionnel. Les asthmes « mixed granulocytiques » alliant une inflammation à la fois neutrophilique et éosinophilique présentent une diminution de la fonction respiratoire plus importante au fur et à mesure de l'évolution. Par ailleurs, le type d'inflammation est un critère prédictif majeur de la réponse au traitement que ce soit sur le plan de la réponse aux corticoïdes inhalés ou sur le plan de la réponse aux biothérapies.

Même si le phénotypage inflammatoire de l'asthme est devenu incontournable dans la prise en charge de l'asthme au cours de la dernière décennie, les informations disponibles concernant l'asthme professionnel et le type d'inflammation restent maigres.

2.4 De l'endotype au trait traitable

Nous avons vu que la caractérisation de l'asthme en fonction du type de déclencheur, de la présentation clinique ou du type d'inflammation permettaient de définir des phénotypes d'asthme. Cependant, il a été émis des critiques concernant ces distinctions, notamment du fait de leur caractère biaisé car issues de catégorisation selon des critères à priori. Il a été émis l'hypothèse qu'une vision plus holistique de l'hétérogénéité de la maladie pourrait permettre de mettre en évidence des mécanismes inflammatoires distincts qui pourraient expliquer l'hétérogénéité de la maladie et déterminer des sous-groupes de patients qui seraient déterminés par ces mécanismes ainsi que par la réponse à un traitement spécifique. C'est ce

qu'on appelle un endotype, néologisme apparu en 2008 correspondant la contraction de « endo-phenotype ». Pour identifier des endotypes, des études de clustering non supervisées ont été produites afin d'explorer l'hétérogénéité de la maladie sous toutes ses dimensions. Ces études ont permis de mettre en lumière l'importance de l'âge de survenue de l'asthme, de la fonction respiratoire et du statut allergique dans la catégorisation des patients asthmatiques. Malgré les efforts déployés, ces études n'ont pas permis d'identifier de sous-groupes de patients définis par un mécanisme moléculaire ou par une réponse à un traitement spécifique. En réaction de cette approche holistique, une nouvelle approche de l'asthme est actuellement développée, celle des traits traitables. Il s'agit d'une approche plus réductionniste qui stipule que l'hétérogénéité de l'asthme doit être considérée à l'échelle individuelle, en prenant en compte toutes ses composantes, en ne préjugeant pas de relations de causalité entre elles. 3 types de traits traitables ont été définis : pulmonaire (l'obstruction bronchique, l'emphysème, l'allergie...), extra pulmonaire (comorbidité, dépression, anxiété...) et de facteurs de risque/ style de vie (observance, tabagisme). Cette approche vise à permettre une vision qui encourage une prise en charge multidisciplinaire, et ainsi, éviter les écueils de la prise en charge thérapeutique par palier, qui provoque un surtraitement de l'inflammation bronchique sans prendre en charge des facteurs associés qui sont pourtant traitables. En effet, un trait traitable est défini par trois caractéristiques : 1) être pertinent cliniquement, 2) être détectable, 3) être accessible à un traitement.

Nous avons vu que la classification de l'asthme professionnel a grandement évolué récemment. Il serait donc intéressant de savoir si la caractérisation de l'asthme professionnel en fonction des caractéristiques sus mentionnées pourrait permettre une meilleure caractérisation de la maladie.

3.Phénotypage de l’asthme professionnel

Les expositions professionnelles peuvent induire ou aggraver une pathologie asthmatique. On estime qu’un asthme sur 4 est soit causé soit aggravé par l’activité professionnelle.
L’asthme en lien avec le travail regroupe les asthmes professionnels, induits par le travail et les asthmes aggravés par le travail, correspondant à des patients préalablement asthmatiques qui vont décrire une aggravation de leur maladie à la suite de leur activité professionnelle. La fraction attribuable des expositions professionnels dans la survenue de l’asthme est estimée être de 16%.
L’asthme professionnel est défini comme étant un asthme dont la source ne peut être expliquée par une cause extérieure au lieu de travail, résultant d’un mécanisme immunologique (asthme professionnel par sensibilisation) ou d’un mécanisme non immunologique (asthme induit par les irritants).

3.1 L’asthme professionnel par sensibilisation

Plus de 400 substances ont été décrites comme pouvant induire un asthme. Les agents les plus couramment mis en cause sont les farines (boulangerie), les produits de ménage dont les ammoniums quaternaires, les isocyanates (peinture) et les persulfates (coiffeurs). L’incidence de l’asthme aurait tendance à diminuer ces dernières années, mais cette tendance est à prendre avec précaution au regard du manque de données disponibles.

L’asthme professionnel par sensibilisation est historiquement catégorisé en fonction de la masse moléculaire de l’agent incriminé. Les asthmes induits par des agents de Haut Poids Moléculaire (HPM) dont la masse moléculaire est supérieure à 5kDa, résulteraient d’un mécanisme IgE médié, les asthmes induits par des agents de Bas Poids Moléculaire (BPM) n’ont pas de mécanisme définitif mis en évidence pour l’instant. Des différences

phénotypiques en fonction de cette classification empirique ont été mises en évidence. Les asthmes induits par des agents de HPM présentent plus souvent un terrain atopique, une rhinite ou une conjonctivite associée. Les asthmes de BPM eux, présenteraient plus d'exacerbations, plus d'expectoration et plus d'oppression thoracique. Des différences ont aussi été observées sur le plan des facteurs favorisant la survenue d'asthme professionnel par sensibilisation en fonction du type d'agent. Les asthmes induits par les HPM sont favorisés par un terrain atopique, une hyperréactivité bronchique non spécifique, l'exposition à la fumée de cigarette et une rhinite allergique. Ces facteurs de risque n'ont pas été retrouvés pour l'asthme induit par les agents de BPM. Cependant pour tous les types d'asthmes professionnelspar sensibilisation le principal facteur de risque reste l'intensité de l'exposition.

Il existe aussi des différences entre les asthmes induits par des agents de HPM et de BPM en termes de stratégie diagnostique. En effet, pour les asthmes de HPM, il existe des tests immunologiques spécifiques (prick test et sIgE) qui présentent une valeur prédictive positive, d'autant plus grande que le signal est élevé. Ces tests immunologiques ne sont que rarement disponibles pour les asthmes de BPM, et quand ils le sont, ils ne présentent qu'un pouvoir discriminant modéré à faible pour le diagnostic de l'asthme professionnel.

Cette classification comporte des limitations. Notamment, il a été mis en évidence des mécanismes IgE médiés pour certains asthmes professionnels induits par des agents de BPM comme les sels de platines ou les acides d'anhydride. Par ailleurs certains agents de BPM provoquent des asthmes pour lesquels le mécanisme IgE médié n'a pas été prouvé mais pour lesquels la présentation clinique est semblable à celle des asthmes induits par des agents de BPM.

Cette classification ne prend pas en compte le type d'inflammation bronchique de l'asthme, qui est devenu incontournable dans l'asthme non professionnel. De plus il est fort probable

que les asthmes induits par des agents de BPM soient une catégorie hétérogène regroupant des patients présentant des mécanismes physiopathologiques différents.

3.2 L'asthme professionnel par irritation

L'asthme professionnel par irritant est causé par un stimulus non immunologique présent sur le lieu de travail. La première description de cette entité a été faite dans les années 80 et décrivait des patients ayant présenté des symptômes d'asthme à la suite d'exposition massive à une substance irritante alors qu'ils étaient indemnes de toute maladie respiratoire jusqu'alors. Cette entité était caractérisée par le fait que la réexposition à la même substance à des doses non toxiques ne provoquait par de reproduction de la réaction asthmatique contrairement à l'asthme professionnel par sensibilisation. Par la suite il a été décrit, notamment après les attentats du 11/09/2001, la survenue d'asthme après une exposition modérée, survenant après un délai pouvant aller jusqu'à plusieurs mois après la première exposition. En 2014, l'EAACI a publié un *position paper*, déterminait des asthmes par irritation aigue, correspondant à la première catégorie décrite, et des asthmes par irritation probable, correspondant à la deuxième catégorie. Deux mécanismes sont évoqués, la lésion tissulaire, et l'irritation sensorielle. Il n'existe que peu de données sur leur caractérisation phénotypique, sur leur type d'inflammation bronchique ni sur leur devenir à long terme. Les informations disponibles suggèrent une inflammation T2 basse, des sujets présentant un asthme mal contrôlé, peu atopique, et qui auraient un pronostic à long terme plus sombre que celui des asthmes professionnels par sensibilisation.

Par ailleurs, les dernières années ont permis l'avènement d'études épidémiologiques qui ont montré que l'exposition à des doses chronique d'irritants bronchiques pouvait provoquer à

long terme un surrisque de survenue de l'asthme. Les données phénotypiques sont là encore plus rares, mais il semblerait que ces profils soient associés à une fonction respiratoire plus altérée et à un plus haut taux de neutrophiles.

4. Objectif

Nous avons pu constater que la caractérisation de l'asthme et sa classification a largement évolué ces dernières décennies. L'hypothèse de ce travail est que les éléments qui ont été prouvés comme importants pour la caractérisation des phénotypes de l'asthme non professionnel le sont aussi pour l'asthme professionnel. Pour améliorer la caractérisation de l'hétérogénéité de l'asthme professionnel le travail de cette thèse résidait en 4 objectifs :

1) Caractériser les profils cliniques, fonctionnels et inflammatoires de l'asthme induit par un agent spécifique, les Ammonium Quaternaires (AQ)
2) Étudier l'impact de la présence de comorbidités, en particulier la dysphonie liée au travail, dans l'asthme induit par un agent sensibilisant
3) Étudier les caractéristiques cliniques et fonctionnelles de l'asthme induit par les sensibilisants en fonction des schémas inflammatoires des expectorations induites
4) Caractériser l'association entre l'exposition à une profession irritante et les schémas de sensibilisation aux IgE spécifiques dans l'asthme de l'adulte.

5. Méthodes :

Il existe deux types d'approches pour étudier l'asthme professionnel, l'investigation de cas dont la preuve a été faite sur le plan individuel de la causalité de l'exposition dans la survenue de la maladie par la réalisation d'un test de provocation spécifique (TPS) et l'investigation épidémiologique qui consiste en l'étude de l'association entre l'exposition et la maladie sur le plan collectif. Dans ce travail, on a adopté les deux types d'approches pour étudier respectivement l'asthme professionnel par sensibilisation et par irritation.

La cohorte E-PHOCAS regroupe un large nombre (n=1518) de patients pour lesquels a été porté un diagnostic d'asthme professionnel par sensibilisation via un test de provocation spécifique entre 2006 et 2018 dans 20 centres européens. Cette base de données rétrospective regroupe des renseignements socio-démographiques, cliniques, fonctionnels et inflammatoires. L'évaluation de l'inflammation bronchique était déterminée par la réalisation d'expectorations induites.

Il a pu être réalisé l'analyse phénotypique des patients asthmatiques professionnels par sensibilisation en fonction des différents critères d'intérêt mentionnés dans les objectifs. Une analyse comparative des caractéristiques cliniques, fonctionnelles et inflammatoires a été réalisée en fonction de la présence ou non de ces derniers critères. Puis, des analyses multivariées ont été effectuées pour déterminer les caractéristiques indépendamment associées à ces critères.

La cohorte multicentrique française EGEA (Étude épidémiologique des facteurs Génétiques et Environnementaux de l'asthme) est une étude épidémiologique enrichie de patients asthmatiques qui présentent de riches données, notamment sur la caractérisation de la sensibilisation IgE par technique de *microarray*. De plus les expositions professionnelles étaient estimées via une matrice emploi exposition spécifique de l'asthme professionnel.

Il a été étudié l'association entre l'exposition professionnel aux irritants et la sensibilisation IgE. Pour ce faire il a été réalisé une analyse transversale de l'association entre les expositions professionnelles vie entière et la sensibilisation IgE. La sensibilisation IgE était décrite en termes de nombre de sensibilisations et de type de sensibilisation. Les différents types de sensibilisation ont été déterminé par la réalisation d'analyse en classe latente qui permet de regrouper les participants en fonction de la probabilité d'appartenir à une classe donnée. Cette méthode a été appliquée sur le fait d'être sensibilisé à 37 allergènes d'intérêt choisi *a priori*.

6. Résultats

Ammonium quaternaires et asthme professionnel par sensibilisation

Les produits de ménage sont une source connue d'asthme professionnel. La multiplicité des agents dans les produits de ménage rend complexe l'étude de chacun des composés chimiques. Les ammoniums quaternaires (AQ) (substance chimique largement utilisée dans des produits de nettoyage (ex. détergents), des désinfectants et de nombreux produits cosmétiques (ex. shampoings, lotions, dentifrices)) sont de plus en plus utilisés. Le rôle sensibilisant des AQ, suggéré dans de rares exceptions par quelques études précédentes, nécessitait d'être exploré plus avant. Cette étude avait pour objectif de caractériser les patients présentant un asthme professionnel par sensibilisation aux AQ, prouvé par test de provocation bronchique, sur le plan clinique, fonctionnel et inflammatoire.

Via l'étude des fiches de données de sécurité des sujets sensibilisés aux produits de ménage de la cohorte E-PHOCAS, 22 sujets ont été identifiés comme étant sensibilisés uniquement aux AQ. En comparaison par rapport à des sujets présentant un asthme professionnel sensibilisés à des agents de bas poids moléculaire, les patients sensibilisés aux ammoniums quaternaires présentaient un profil inflammatoire plus éosinophilique (expectoration induite post test à 24.2% en médiane contre 4%, p valeur<0.001).

Dysphonie au travail et asthme professionnel par sensibilisation

La dysphonie est surreprésentée dans la population asthmatique, essentiellement d'origine fonctionnelle. Il a été décrit des tableaux de dysfonction laryngée induite par le travail pouvant coexister avec un asthme professionnel. L'objectif de cette étude était de caractériser sur le plan socio-démographique, clinique et inflammatoire les participants avec asthme professionnel qui

rapportaient une dysphonie au travail. Cette étude a permis d'estimer que chez les patients issus de la base de données E-PHOCAS avec des données renseignées sur l'expectoration induite et la dysphonie (n=341), 14% présentaient une dysphonie au travail. Cette dysphonie était associée au genre féminin et à une inflammation neutrophilique. Cette étude, est la première à avoir montré une association entre la dysphonie au travail et l'inflammation neutrophilique

Inflammation bronchique et asthme professionnel par sensibilisation

La détermination du phénotype inflammatoire dans l'asthme permet de distinguer les patients en fonction de caractéristiques cliniques et est prédictive de la réponse à la corticothérapie. Dans l'asthme professionnel par sensibilisation, les rares études qui ont décrit les profils inflammatoires des patients suggèrent que ces profils ne diffèrent pas selon la masse moléculaire de l'agent causal. L'objectif de ce travail était de déterminer si, dans l'asthme professionnel, le type d'inflammation bronchique était associé à des caractéristiques cliniques distinctes, et au type d'agent causal.

Dans cette étude, basée sur 296 patients, l'analyse des expectorations induites identifiait majoritairement une inflammation éosinophilique (67,9%). L'éosinophilie bronchique était associée à une pression thérapeutique plus grande, et à une hyperréactivité bronchique spécifique plus sévère. L'inflammation neutrophilique était associée à un plus mauvais contrôle, à plus d'exacerbations, au genre masculin et à un âge plus avancé. Les différents types d'inflammation éosinophilique et neutrophilique étaient associés à des caractéristiques cliniques et fonctionnelles indépendamment du type d'agent causal. Cela souligne l'importance du phénotype inflammatoire dans l'asthme professionnel par sensibilisation. De plus il a été montré que pour un même type d'agent causal, plusieurs types d'inflammation bronchique pouvaient être observés.

Exposition professionnelle aux irritants et sensibilisation IgE dans la cohorte EGEA

Les expositions professionnelles aux irritants ont été décrites comme associées à un asthme non atopique dans des études antérieures. D'autres études bénéficiant d'une caractérisation approfondie de la sensibilisation allergique nécessitent d'être conduites pour évaluer plus avant cette association. La caractérisation de la sensibilisation IgE via la technique des micro-puces a permis d'accroitre la compréhension des allergies croisées et la caractérisation de profils de sensibilisation, associée à des paramètres de santé respiratoire.

L'objectif de l'étude était d'étudier l'association entre les expositions professionnelles aux irritants et la sensibilisation IgE médiée, mesurée par la technologie des micro-puces, chez les participants avec et sans asthme.

Sur la base des données collectées dans la cohorte EGEA, 924 participants avec des données renseignées concernant les expositions professionnelles et la sensibilisation IgE ont été sélectionnés. Les résultats de l'étude ne mettaient pas en évidence d'association entre l'exposition professionnelle aux irritants et la sensibilisation IgE chez les participants non asthmatiques et ceux ayant présenté un asthme dans l'enfance. Cependant, chez ceux qui avaient débuté leur asthme à l'âge adulte, une tendance était retrouvée entre l'exposition professionnelle aux irritants et une moindre sensibilisation IgE et en particulier vis-à-vis des acariens. Il pourrait être fait l'hypothèse que les sujets ne présentant pas de sensibilisation IgE soient plus vulnérables à la survenue d'asthme induit par les irritants.

Ces résultats limités par le design transversal de l'analyse principale, ne permettent pas de définir le sens de l'association entre l'exposition professionnelle aux irritants et la sensibilisation IgE. Ils soulignent la nécessité d'études longitudinales afin de mieux caractériser le rapport entre l'exposition aux irritants et l'incidence de nouveaux cas d'asthme en fonction de caractéristiques propres au patient.

7. Discussion générale

L'asthme professionnel a été décrit comme un phénotype spécifique de l'asthme. Force est de constater, au vu de nos résultats, que c'est un ensemble hétérogène tout comme l'asthme l'est par ailleurs. Ces résultats devraient nous permettre d'améliorer la prise en charge de l'asthme professionnel de façon plus précise. Cependant plusieurs questions restent en suspens. Tout d'abord nous avons observé que l'asthme professionnel par sensibilisation aux agents de HPM et de BPM étaient l'un comme l'autre associés de façon prédominante à une inflammation éosinophilique. Pour ma part je ne pense pas que l'on puisse conclure de cette information qu'ils relèvent du même mécanisme physiopathologique car nous avons vu auparavant que l'inflammation éosinophilique pouvait survenir en réponse à divers stimuli. De plus nos résultats révèlent qu'un agent donné pouvait induire différents types d'inflammation. Cela supporte à mon sens le fait qu'il faille tenir compte de l'hétérogénéité des patients sur un plan individuel comme le recommande la méthode des traits traitables. De plus il est probable que l'asthme professionnel ne recèle pas en son sein de mécanisme qui lui serait spécifique. Par exemple l'asthme allergique ou induit par les irritants sont aussi retrouvés dans l'asthme non professionnel. Cependant il me semble crucial d'étudier l'asthme en lien avec le travail car il est pourvoyeur d'un fardeau important tant sur le plan sanitaire et social, qui touche les personnes les plus fragiles.

La force de ce travail de thèse réside en l'utilisation à la fois d'une cohorte spécifique de l'asthme professionnel par sensibilisation et d'une étude épidémiologique enrichie avec des cas d'asthme. Concernant la cohorte E-PHOCAS son caractère rétrospectif n'a pas pu permettre d'asseoir des trajectoires en fonction des phénotypes identifiés. De plus, les méthodes de réalisation d'expectoration induite n'étaient pas les mêmes dans tous les centres, provoquant une variation intercentre. Pour la cohorte EGEA, le caractère rétrospectif n'a pas permis de

déterminer de conclusion solide sur le sens de l'association entre les expositions professionnelles et la sensibilisation IgE médiée.

Dans le futur il serait intéressant de réaliser une étude prospective longitudinale à partir de la cohorte E-PHOCAS pour déterminer l'apport prédictif de l'éosinophilie bronchique dans le devenir à long terme de l'asthme professionnel. Il pourrait être fait aussi un état des lieux des autres traits traitables notamment des comorbidités. Concernant les asthmes induits par irritation il serait souhaitable de réaliser une étude longitudinale pour déterminer la fraction attribuable spécifiquement liée à l'exposition aux irritants, et pour étudier les effets modulateurs de l'association entre expositions aux irritants et la survenue de cas incidents d'asthme professionnel par irritation.

8. Conclusion

Les résultats des travaux de la thèse indiquent que l'asthme professionnel, loin d'être un ensemble homogène, est constitué d'un ensemble de phénotypes cliniques et inflammatoires. La dichotomie historique de l'asthme professionnel par sensibilisation en asthme professionnel sensibilisé à des agents de haut et bas poids moléculaire ne semble pas suffisante pour pouvoir tenir compte des différences phénotypiques observées. À l'instar de l'asthme non professionnel, la caractérisation des patients en fonction du type d'inflammation bronchique et en fonction des formes cliniques semble être importante. Par ailleurs, nos résultats montrent que l'exposition aux irritants est associée à une moindre sensibilisation IgE, chez les personnes présentant un asthme de l'adulte. Cela renforce l'idée que l'asthme induit par les irritants se développe indépendamment de mécanisme IgE médiés contrairement aux asthmes professionnels par sensibilisation. Grâce à une meilleure caractérisation phénotypique de l'asthme professionnel, ces travaux de thèse pourraient contribuer à mettre en place des prises en charge thérapeutiques plus précises, adaptées aux différents phénotypes.

Nicolas MIGUERES

PHENOTYPES DE L'ASTHME PROFESSIONNEL

Dans le cadre des cohortes E-PHOCAS et EGEA

Résumé

L'asthme est une maladie dont l'hétérogénéité a été démêlée par la caractérisation de phénotypes. L'asthme professionnel est un type d'asthme lié au travail, qui est causé par des stimuli immunologiques (allergique) et non immunologiques (asthme induit par des irritants) présents sur le lieu de travail. Afin de mieux comprendre les phénotypes de l'asthme professionnel, ce travail de doctorat visait à caractériser l'asthme professionnel en termes d'agent causal, de comorbidité et de profil d'inflammation. Les analyses ont été effectuées dans la cohorte E-PHOCAS, qui rassemble des patients atteints d'asthme professionnel identifiés par un test de provocation spécifique, et dans l'étude EGEA, une cohorte épidémiologique enrichie de participants asthmatiques. Il a été démontré que des éléments clés de la caractérisation de l'asthme non professionnel, tels que le profil inflammatoire et les comorbidités, permettent également de distinguer des phénotypes de l'asthme professionnel. En outre, il a été observé qu'un agent professionnel sensibilisant pouvait induire un asthme professionnel par différentes voies inflammatoires. Ces résultats remettent en question la classification historique de l'asthme professionnel qui repose exclusivement sur le type d'agent incriminé.

Mot clefs : asthme professionnel, phénotype

Résumé en anglais

Asthma is a disease whose heterogeneity has been detangled by the characterization of distinct phenotypes. Occupational asthma is a type of work-related asthma, that is caused by immunological (i.e sensitizer induced asthma) and non-immunological (i.e irritant induced asthma) stimuli present in the workplace. In order to provide better insights in occupational asthma phenotypes, this PhD work aimed to characterize occupational asthma in term of offending agent, comorbidity and inflammatory pattern. Analyses were performed in the E-PHOCAS cohort which gather occupational asthma patients ascertained by a specific inhalation challenge and in EGEA study, an epidemiological cohort enriched with participants with asthma. It has been shown that key elements of the characterization of non-occupational asthma such as inflammatory pattern, comorbidities also distinguish phenotypes in occupational asthma. Moreover, it was observed that a sensitizing occupational agent could induce occupational asthma through different inflammatory pathways. These results challenge the historical classification of occupational asthma which relies exclusively on the type of the offending agent.

Key word: occupational asthma, phenotype