Pathogenesis, clinical features of asthma COPD overlap (ACO), and therapeutic modalities

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Abstract

Both asthma and COPD are heterogeneous diseases identified by characteristic symptoms and functional abnormalities, with airway obstruction common in both diseases. Asthma COPD overlap (ACO) does not define a single disease but is a descriptive term for clinical use that includes several overlapping clinical phenotypes of chronic airways disease with different underlying mechanisms.

This literature review was initiated to describe published studies, identify gaps in knowledge, and propose future research goals regarding the disease pathology of ACO, especially the airway remodelling changes and inflammation aspects. Airway remodelling occurs in asthma and COPD, but there are differences in the structures affected and the prime anatomic site at which they occur. Reticular basement membrane thickening and cellular infiltration with eosinophils and T-helper (CD4+) lymphocytes are prominent features of asthma. Epithelial squamous metaplasia, airway wall fibrosis, emphysema, bronchoalveolar lavage (BAL) neutrophilia and (CD8+) T-cytotoxic lymphocyte infiltrations in the airway wall are features of COPD. There is no universally accepted definition of ACO, nor are there clearly defined pathological characteristics to differentiate from asthma and COPD. Understanding etiological concepts within the purview of inflammation and airway remodelling changes in ACO would allow better management of these patients.

Key Words

Asthma, COPD, asthma-COPD overlap (ACO), airway remodelling, inflammation.

1.0 Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are chronic airway diseases with variable expiratory airflow limitations. Both diseases are associated with airway remodelling and chronic inflammation. However, the nature and site of inflammation differ between diseases and within the diseases themselves, resulting in different pathogenic mechanisms and clinical phenotypes, implying that they would also require different strategies for treatment (15, 19, 148, 154). At the Bronchitis Symposium, at Groningen, the Netherlands (the year 1960), Professor Orie and colleagues articulated an aetiological hypothesis under which asthma and COPD (then labelled as bronchitis) were suggested to have shared origins and clinical expressions. The disparity in the pathology was based on genetic information and environmental exposures, and the term "chronic non-specific lung disease" (CNSLD) initially described mixed pathological conditions. Later, this hypothesis was termed the "Dutch Hypothesis" by Fletcher and Pride (155) and referred to as such. Despite the influential paradigm of the "Dutch hypothesis", opponents have vehemently opposed it (13, 208). In clinical practice, patients with asthma and COPD are treated under the "rubric of a fixed dichotomy" of separate diseases (123). The opposing "splitting view", also known as the "British hypothesis", proposed that asthma and COPD do not have a common origin and are perceived as distinct disease entities generated by different mechanisms caused by distinct pathogenesis. Over the years, considerable progress has been made in understanding the airway inflammatory (both innate and adaptive) cells driving airway pathology of asthma and COPD. Within these aspects, the current broad consensus is that asthma is majorly driven by a type 2 helper T Cell (Th2) response while COPD is biased more towards type 1 helper T Cell (Th1) phenotypic response.

Asthma involves increased activity and infiltration of innate cells such as eosinophils and mast cells along with extensive airway remodelling, in particular reticular basement

membrane (Rbm) thickening and smooth muscle hypertrophy. On the other hand, COPD includes neutrophils and macrophages and small airway remodelling such as small airway wall fibrosis and squamous metaplasia (49, 83, 97, 104). Although these two diseases are viewed as clinically different entities, some patients share several overlapping features of asthma and COPD. Acknowledging this reality, both Global Initiative for Asthma (GINA) and COPD (GOLD) documents introduced the term asthma-COPD overlap syndrome (ACOS) to describe the existence of this patient cohort (69). However, this terminology is controversial since it is not representing a single disease entity (211); therefore, the term 'asthma-COPD overlap (ACO)' according to the current GINA and GOLD guidance has been used in this review.

Currently, it is estimated that about 30% of COPD and 26% of asthma patients have symptoms associated with ACO (21, 65, 88). Studies indicated that the ACO patients have an earlier onset of disease compared to COPD patients (39), and is more common in females than in males (205), more prevalent with individuals in lower socioeconomic status, and affects those with existing comorbidities (114). Exacerbation rates, emergency department visits and hospital admissions are also higher among ACO patients (61, 132), affecting the overall per capita healthcare cost (160). Despite the sizeable clinical implication, it is surprising that no consensus to a universally accepted definition or a clear diagnostic criterion for ACO exists. Moreover, excluding ACO patients from asthma and COPD clinical trials created inadequate evidence-based treatment regimens for these select group of patients (31, 153). It is thus imperative that the disparity in the overlap epiphenomenon of asthma and COPD is critically analysed. Primarily, ACO patients' can be characterized by unravelling the inflammatory and remodelling processes involved in the lung compartments of these patient groups. A more thorough understanding can help develop better diagnoses and new therapeutic approaches to this disease. This review provides a comprehensive overview of the

existing literature on ACO pathology as a clinical phenotype, current therapeutic management, pathologically distinguishing similarities and differences in asthma, COPD, and ACO patients.

2.0 Asthma COPD Overlap

The asthma COPD overlap (ACO) is not a single disease entity (2, 3) and collectively describes the patients who have persistent airflow limitation and clinical features consistent with asthma and COPD. The ACO phenotype has been a matter of immense concern as most clinical trials exclude these patients, causing a paucity of evidence that leads to overtreatment, especially with inhaled corticosteroids (ICS), which could be more damaging than beneficial. The ACO phenotype remains undefined (211); the prevalence is also considerably variable, 0.9 to 11% in the general population, 11.1% and 61.0% in the asthma patients, and 4.6 to 66% in the COPD patients (2, 203). Even the criteria mentioned in guidelines for the diagnosis of ACO patients do not align among themselves. However, similarities exist among the essential aspects: the persistent airflow obstruction is consistent with COPDs with a history of asthma diagnosed before and after 40 years of age, and also the positive bronchodilator responsiveness, i.e., improvement of FEV_1 by at least 15% and 400 ml against the pre-bronchodilator value (200). A global survey by Jenkins C et al. (100) among the respiratory-allergy specialists and primary care practitioners found that patients with a history of asthma, allergy/atopy, smoking and toxic exposure or with respiratory symptoms such as dyspnoea, chronic cough, and chest tightness were primarily used for diagnosis and management of ACO. The ACO shares many risk factors established for asthma and COPD and many of these overlapping factors could have early origins in the disease. Also, over time the clinical signs and symptoms of asthma and COPD become similar due to prolonged exposure to environmental hazards such as cigarette smoking, smoke generated from burning of fossil fuel, and the very chronic nature of these diseases.

However, a prominent phenotype of one disease still be observed in these patients (131, 138, 154).

2.1 ACO Pathology

The Debate

A common debate on ACO pathogenesis is whether it is a unique pathogenic process as suggested by the British hypothesis or a synergistic pathological process of asthma and COPD as in the Dutch hypothesis. Neither of these two theories has sufficient evidence to prove or invalidate the mystery of ACO. Evidence suggests that the ACO is a specific treatable trait with a Th2 signature expressed by the blood eosinophil as a biomarker (202). Taken together, we believe that the ACO is a clinical phenotype that lies within the prism of both the "Dutch" and "British" theories and is undoubtedly different from those with either COPD or asthma. A well-designed study by Ghebre et al. (67) illustrated this point while investigating the predominant overlapping pathobiological characteristics by comparing spontaneous or induced sputum inflammatory mediators from ACO patients. Based on their clustering analysis, they identified 3 biological clusters. In the Cluster 1, there was dominance of Th2 cytokines and eosinophils with 95% in asthmatics and 5% in patients with COPD. Cluster 2 was identified with neutrophilia and high cytokine IL-1β in asthmatic COPD overlap patients, closer to the Dutch theory. COPD with mixed granulocytic airway inflammation and higher sputum IL-6 and CCL13 levels were identified in Cluster 3. These findings indicated the different origins of asthma and COPD, as stated by the British hypothesis. Nonetheless, the study had a shortcoming in reporting the bacteriological data, was exclusively based on culture-dependent methodologies and lacked supporting serological data.

Asthma or COPD to ACO Process

The airway inflammatory patterns in asthma and COPD are distinct. A systemic inflammatory network analysis indicated a mixture of asthma and COPD inflammatory pattern in ACO with Th2 (IL-13 and IL-5) and non-Th2 cytokine expression (44). The study reported that the median values of IL-13 and IL-5 were highest in asthma, intermediate in ACO, and lowest in COPD. Interestingly, IL-8 was higher in patients with a smoking history (COPD and ACO) than asthma (44), attracting neutrophils and macrophages. Conventionally, asthma is viewed as a disease of variable airflow obstruction due to allergen exposure with airway hyperresponsive. Thus, it is easy to perceive that an asthmatic person who smokes tobacco eventually develops a s fixed airflow obstruction due to increased inflammatory response and consequent COPD (Figure 1).

Sputum and endobronchial biopsy studies have revealed that smoking in asthma increases airway neutrophilia, a pattern similar to COPD, presumably by expressing cytokines such as IL-6, IL-8, and IL-17A (71, 156, 173). These cytokines have been implicated in neutrophil chemotaxis in smokers with asthma. In addition, IL-17 plays a crucial role in bronchial asthma driven by neutrophilic inflammation (173) and is also likely involved in stimulating MMP-9 secretion from macrophage in COPD (133). Further, a previous study by Ravensberg et al. (157) in airway pathology of smoking asthmatics found an increase in bronchial infiltration of CD8+ T cells, macrophages and epithelial remodelling akin to COPD. Interestingly, no difference was observed in neutrophil numbers when compared to non-smoking asthmatics, thus suggesting that CD8+ and macrophages are the dominant inflammatory cells in smoking asthmatics. Besides smoking, air pollution also affects the asthmatic airway in numerous ways, including increasing cellular oxidative stress, cytokine/chemokine release, innate immune cell activity through damage-associated

molecular pattern (DAMP) receptors such as TLR-2 and TLR-4, regulatory T-cells dysfunction, and alterations in DNA methylation (123).

Abundant literature evidence is available for the single-nucleotide polymorphisms (SNPs) in asthma gene candidates. However, replication of these genes has been an issue, and only a few were replicated *TNF, ADAM33, IL-4RA, MMP9, IL-12B, C3*, and *IL-13* (204). Further, genome-wide association studies (GWAS) of asthmatic adults and children have identified asthma with SNPs of *GATA3, MUC5AC, KIAA1109, HLA-DR, IL-33, IL-1RL1IL-18R1, SMAD3, ORMDL3/GSDMB,* and *IL-2RB* (137, 172). The locus *ORMDL3/GSDMB* on chromosome 17q21 is specific to the early-onset of asthma. The asthma exacerbation in ICS treated children is associated with *APOBEC3B* and *APOBEC3C* (77, 137). These genes are implicated in innate and adaptive immune responses and anomalies of epithelial barrier function in asthma. For example, the possible regulatory role of *SMAD 3* and *IL-2RB* in the homeostatic and healing process and thus could have potential role in airway remodelling.

Another important gene in asthma is GSTP1, the most abundant isoform of the GST gene in the lung epithelium. Genetic polymorphisms of this gene have been implicated in asthma pathogenesis. For example, GSTP1-105 polymorphism has been reported as a predictor for asthma in Taiwanese school children (120); GSTP1 Ile105Val susceptibility in childhood asthma in the Japanese population (109). Nonetheless, the GSTP1 homozygous Val/Val genotype was also associated with a 3.6-fold increased risk of having asthma in Turkey (199). Interestingly, findings by Piacentini et al. (151) questioned the sustainability of association of this gene with asthma as the author did not find any significant differences in the genotype distributions GSTP1 along with of the GSTM1 and GSTT1 genes between asthmatics and healthy controls from Italy. Overall, these findings remind the ethnicity factor in the genetic polymorphism.

One of the proven genetic determinants of COPD is severe alpha 1-antitrypsin (*AAT*) deficiency. The causative relationship between defective one single gene *AAT* (mutation of the *SERPINA1* gene) and the development of pulmonary emphysema was first noted back in 1963 (118). The *AAT* deficient patients with protease inhibitor (PI) type Z allele are at increased risk for severe, early-onset COPD (117). The COPD GWAS have identified and replicated SNPs at chromosome 15q25 spanning many genes, including *CHRNA3-5* and *IREB2* as the potential candidate in COPD susceptibility. Further, the *HHIP* locus is associated with fat-free mass, exacerbations among COPD patients, and FEV_1/FVC (152). The CHARGE Consortium also found evidence of association of *FAM13A* locus with FEV_1/FVC (209). Another identified genetic defect linked with COPD is the *TERT* gene mutations, a risk factor for emphysema in smokers predominantly female and approximately 1% severe COPD had this deleterious mutation in *TERT* (198).

The potentially shared genetic risk factors for asthma and COPD included *TGFB1, TNFA, MMP9, GSTP1, IL-13, SERPINE2, SOX5, WNT5a,* and *DDX1* (11, 75, 78, 178, 206). Further, *ADAM33* has been linked to both diseases and accelerated lung function decline (105). Thus, the shared gene theory suggested a common underlying genetic factor for both the onset and course of these two diseases. In addition, among the shared genes, *ADMA33, SERPINE2, SOX5*, and *MMP9* are important for lung development and injury repair (89).

The genetic work in ACO is at an early stage. Hardin et al. (75) GWAS study of asthma and COPD found no SNPs associated with ACO exceeding a significance level (p-value) of 5×10^{8}. However, the most significant variant was in the *CSMD1* gene on chromosome 8, followed by the intronic region in the *SOX5* gene on chromosome 12. The top two SNPs (rs11779254 and rs59569785) associated with ACO were significant among the non-Hispanic (n=283) but in the African-American (n=167) due to small sample size. The meta-analysis identified the association between SNPs in the gene *GPR65* (member of G2A G protein-

coupled receptor family) and ACO. Gene *GPR65* plays a crucial role in eosinophil activation during asthma and extracellular inhibition of proinflammatory cytokines. Recently, Joo et al. (106) identified 24 loci associated (p-value < 5e-8) with ACO, including well-known asthma and COPD loci such as *ORMDL3/GSDMB* and *HHIP* in a GWAS from UK biobank (502456 individuals aged 37 to 73 years). The genome-wide significant loci in ACO (not in asthma or COPD GWAS) included two near the *HRNR* and *ID2* genes, and each of these peaks was nominally associated with asthma (p-value < 1e-6), suggesting that they are risk factors for ACO. Another large GWAS by John et al. (102), including 8068 cases and 40360 controls of European ancestry from UK Biobank and other 12 additional cohorts, identified an intergenic signal on chromosome 5, rs80101740 previously associated with asthma, COPD, or lung function. The author also identified eight genome-wide signals for ACO with the nearest gene *GLB1*, *IL17RD, FAM105A, LOC100289230, TSLP, C5orf56, HLA-DQB*, and *PHB*. Overall, these findings contribute to understanding of genetic overlap between ACO and contributing diseases asthma and COPD.

Allergen sensitization has been reported in elderly COPD patients, and the presence of high-level serum IgE possibly causing allergic inflammation and associated symptoms in COPD (93). Further, cigarette smoking increases the total serum IgE levels; however, it decreases with increasing age. High IgE serum levels were detected in ACO patients compared to COPD (107, 145). Clustering analysis of Th2-gene signatures (*POSTN*, *SERPINB2*, and *CLCA1*) suggest that a more significant subgroup, approximately 20%, of COPD patients with a smoking history had high Th2 signatures (36). However, the asthma-like Th2-associated signatures are not predicted clinically by the history of asthma. The group also found that the Th2 signature in COPD was associated with tissue eosinophilia, blood eosinophilia, high bronchodilator responsiveness and a better response to ICS. Recently, Mertens et al. (134) reported the effect of IL-13 and whole cigarette smoke on the Th2-gene

expression *POSTN*, *CLCA1*, and *SerpinB2* in primary bronchial epithelial cells by using air-liquid interface cultures to explore the underlying mechanism of ACO. The study found that cigarette smoke inhibits IL-13 induced Th2-gene signature, especially the *POSTN* in primary bronchial epithelial cells.

Recently Lange et al. (116) demonstrated a pathway of the inadequately developed lung for the genesis of COPD. The study included subjects from three independent cohorts according to lung function at the beginning and the presence or absence of COPD towards the end. The authors noted a significant mean decline in FEV_1 of 17±18 ml per year in half of the population who had a low FEV_1 in early adulthood, suggesting the lung function values reached during early adulthood are essential for diagnosing COPD later in life.

Overall, we can infer that many shared genes in asthma and COPD are identified in general populations associated with maximally attained lung function, which might explain the commonality rather than shared pathogenesis in asthma and COPD as stated in the Dutch hypothesis.

2.2 Distinguishing ACO from Asthma and COPD

The crucial components of asthma and COPD disease include chronic but variable inflammation throughout the airway and link to airway wall remodelling. Therefore, these two components may also be active in the ACO.

2.2.1 Inflammation

The “Hygiene Hypothesis” of asthma pathogenesis asserts that the childhood exposure of allergens has a protective effect in the development of atopy, possibly by stimulating the Th1 immunity (interleukin [IL]-2, interferon-γ and tumour necrosis factor [TNF]-α mediated) (144). In asthmatic individuals, interactions with infectious agents or allergens through macrophage and dendritic cells stimulate the proinflammatory thymic stromal

lymphoproteins (TSLPs) family of cytokines, activating a more poignant adaptive immune response (60). Atopic asthma individuals have inflammation largely orchestrated by CD4+ cells of T-helper type 2 (Th2) cells (Figure 2). Th2 cells release inflammatory cytokines IL-3, IL-4, IL-5, IL-9, and IL-13, which triggers IgE synthesis in B cells and stimulate recruitment of basophils, mast cells, and eosinophils, their differentiation maturation and survival (16, 162). Further, group 2 innate lymphoid cells (ILC-2 cells), which produce Th2 cytokines such as IL-5 and IL-13, also contribute to eosinophilic inflammation (76). A typical inflammatory pattern also existed in non-atopic asthma wherein similar increases in eosinophils, mast cells, type 2 cytokines IL-4, IL-5, IL-9 and IL-13, and IL-4 receptor-expressing cells (18). Also, epithelial damage due to microbes or pollutants causes induction of 'alarmins' cytokines such as IL-25, IL-33, and TSLP, which again promote eosinophilic inflammation, even in the absence of allergic stimuli (162). Contrarily, Th2 low asthmatic inflammation is driven through Th1/type 17 helper T (Th17) or ILC3 cells response and are found in the presence or absence of neutrophilia (115, 174). The neutrophilic asthma is also linked to IL-17 pathways, (173) essentially producing chemoattractant (CXCL)-8 (IL-8), that attracts a large number of neutrophils at the site of inflammation (161). Lastly, IL-6 and IL-17 promote dual Th2 and Th17 cell phenotypes in mixed granulocyte asthma, wherein eosinophilic and neutrophilic asthma acts concertedly (92). Although it is now known that inflammation induces airway hyperresponsiveness (AHR), the exact mechanism between inflammation and AHR remains unclear (34). The AHR could result from epithelial damage likely through loss of barrier function, peptide inflammatory mediators degrading enzymes such as neutral endopeptidase, epithelial relaxant factor, and exposure of reflexing sensory nerves in the airways (14).

Both innate and adaptive immune responses are involved in lung inflammation in COPD patients (Table 1). The progression of COPD increases with the increasing infiltration of the airways by inflammatory cells (80). Chronic exposure to cigarette smoke, air pollutants, and

biomass fuel directly activate the innate immune response by triggering TLRs or purinergic receptors. Subsequently, in the effector phase, proinflammatory cytokines and chemokines such as TNFα and chemokine CXCL8 are released by airway epithelial cells (Figure 3). Further, the expression of adhesion molecules on endothelial cells promotes the recruitment of macrophages and neutrophils to the lungs (26). Neutrophils release several serine proteases, which act on elastin, collagen, and fibronectin, affecting several clinical facets of COPD, including alveolar destruction. Further, these proteases also enhance the mucus secretion from submucosal glands and goblet cells (17). An increase in macrophage numbers in the airway lumen and airway wall has been established in smokers and COPD patients (51). Although mast cells are traditionally associated with atopic asthma, evidence indicates that mast cells may be implicated in COPD pathogenesis as shown by us and others (7, 192). The activation of the adaptive response starts later in the COPD disease course with the increase of T and B lymphocytes (20). CD8+ cells are predominant cells compared to CD4+ cells in the lungs and arteries of COPD smoker patients (53, 164). Although the CD4+ cells are involved in COPD pathogenesis, the information on the precise involvement of CD4+ cells are still evolving, and a possible role the involvement of cytokines IL-17 and IL-21 in this mechanism has been identified (214). Elevated airway eosinophilic inflammation is present in about 20%–40% of COPD cases despite treatment with ICS (166). The mechanisms of eosinophil elevation in COPD patients are likely to involve ILC2, possibly regulated by the IL-33, released due to epithelial cell injury (17). Oxidative stress, a COPD feature, occurs when exposure to free radicals or reactive oxygen species (ROS) overcomes the defence. The lung is continuously exposed to these ROS generated from exogenous sources such as air pollutants and cigarette smoke, from endogenous sources, e.g., mitochondrial respiration and the inflammatory responses to viral and bacterial infections. The airway epithelial cells have been shown to induce the production of mitochondria-

derived ROS when exposed to the lipophilic part of cigarette smoke. ROS activate NF-κB and P38 MAPK, activating multiple inflammatory genes and proteases and may cause increased inflammatory response (17, 159).

Periostin, an extracellular matrix protein of the fasciclin family and mammalian chitinase-3-like protein 1 (*CHI3L1*) or YKL-40 glycoprotein (produced by various cell types, including macrophages, neutrophils, and airway epithelium) were found to play a vital role in the pathogenesis of airway inflammation, remodelling of tissue in asthma and COPD, respectively. Efforts have been made to differentiate ACO from asthma and COPD by assessing serum periostin and YKL-40 together (171); however, both of these markers are not consistently upregulated in asthma or COPD and are not the representatives of entire asthma and COPD populations. Therefore, the combined assessment of these two biomarkers alone may not be enough (95). Club cells are believed to play an important role in airway repair due to injury, secreting anti-inflammatory, and immunomodulatory proteins. Club cells secretory protein (CC-16) was significantly low in ACO patients, especially with higher smoking levels and airflow obstruction than asthma or COPD patients, suggesting severe inflammation and poorly controlled disease. However, the study did not include the normal healthy controls and may have been confounded with selection bias (143).

Sputum biomarker studies have provided the most specific information on ACO, asthma, and COPD (Table 1). For example, Gao et al. (63) found a higher level of neutrophil gelatinase-associated lipocalin (NGAL), IL-6, and YKL-40 in ACO patients identified using the GINA and GOLD guidance documents as compared to healthy subjects. Only NGAL could differentiate the ACO patients from asthma and COPD. The neutrophil percentage was highest among the inflammatory cells in ACO compared to asthma, COPD, healthy or non-smoker subjects. In another study, sputum neutrophil count was higher in COPD patients than

asthma and ACO, whereas the eosinophils were higher in asthma and ACO than the COPD patients without any apparent differences in eosinophils in between asthma and ACO. However, the sensitivity and specificities were different for the cellular markers (64). A study by Kitaguchi et al. (112) found that the COPD patients, alongside a history of asthma with a thickened bronchial wall, high peripheral and sputum eosinophils had a significant increase in FEV_1 when treated with ICS. Thus, COPD patients with asthmatic symptoms with a high eosinophil count and thickened bronchial wall are more likely to respond to ICS. Interestingly, a high peripheral eosinophil concentration with elevated IL-4 was observed in firefighters with no previous history of asthma , from the dust exposure during the World Trade Center collapse, and were subsequently diagnosed as ACO, indicated through pulmonary function test (PFT) with BDR (FEV_1 increase of >12% and 200 mL from baseline and FEV_1/FVC ratio <0.7) (176). A high sputum eosinophil count was also found by Iwamoto et al. (94) in both asthmatic and ACO patients as compared to COPD and healthy subjects in a study evaluating inflammatory and lung-injury related biomarkers in these patients. The patients in this study were diagnosed in accordance with British Guidelines on Asthma Management and American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations. Currently there is scant evidence with regards to specific biomarkers for ACO, which suggests the possibility of variable inflammatory mechanisms across ACO patient population.

Recently, Ghosh N et al.(68) established a comprehensive serum immunological profile using a combination of gas chromatography and mass spectrometry for asthma, COPD, and ACO patients diagnosed using GINA and GOLD, 2014 and NHLBI/ATS, California Workshop 2016 (1, 210, 211). They identified TNFα, and IL-1β, among the Th1 mediated cytokines and IL-5 as Th2 cytokine that was significantly upregulated in ACO, suggesting that these factors could distinguish asthma and COPD from the former. In addition, the study found an

increased presence of IL-6 expression in ACO, which was suggested as a possible diagnostic biomarker. Further, considering the elevated IL-5, the authors hinted at the possibility of using anti-IL-5 therapy similar to severe asthma as a treatment strategy for ACO.

So far, only one endobronchial biopsy study has been conducted in ACO, asthma, and COPD patients, comparing the histological differences (147). In the study, COPD and asthma patients were diagnosed according to GOLD and GINA criteria and ERS/ATS guidelines, and ACO patients fulfilled the criteria in the published consensus documented by Sin et al. (175). All patients included were under ICS or long-acting β-agonists (LABA) treatment. The study did not find any difference in tissue lymphocyte infiltration, eosinophilic infiltration, number of granulocytes among COPD with or without asthma characteristics. However, asthma only patients had higher tissue eosinophils, and COPD only patients had higher granulocytes in the stroma.

2.2.2 Airway Remodelling

Airway remodelling refers to the structural changes in the airways of many chronic lung diseases, including asthma and COPD. In asthma, they occur in the mucosal areas of the airways with aberrant modifications of both epithelial and subepithelial areas, the smooth muscle layer and airway vascular structures. Structural changes in COPD generally involve thickening of large and small airways and parenchymal alveolar areas, with significant tissue morphological and physiological changes seen such as epithelial metaplasia, loss of epithelial cilia, a high number of goblet cells, and gland and smooth muscle hypertrophy and hyperplasia.

Epithelial Alteration

The bronchial epithelium is considered the frontline protective layer against toxic substances and microbes during inhalation and is crucial in maintaining tissue homeostasis. Any

imbalance in homeostasis accentuates inflammatory response and repair process that help mend the damage.

Shedding the damaged epithelial surface in the asthmatic airway has been reported, although with high variability between cases (98) and without noticeable ciliary abnormalities (99). In general, shedding of epithelium is not reported in COPD; however, cigarette smoking damages the epithelium leading to squamous metaplasia. Increased goblet cells and loss of cilia are other important observations in COPD epithelial areas (80, 98, 165, 190). Goblet cells hyperplasia in COPD patients leads to airway lumen blockage due to increased mucus secretion and plug formation. At the same time, the reduction in cilia length would further disrupt the mucociliary clearance, retaining the mucus within the airway (122). In COPD patients, injury to the epithelium attracts inflammatory cells that alter cellular permeability. Further, altered epithelium increasingly produces growth factors such as TGFβ, epithelial growth factor (EGF) and vascular endothelial growth factor (VEGF) that can cause both physiological and genetic changes (56, 103, 194, 196).

Minimal evidence is available concerning epithelial remodelling changes in ACO patients. However, lately, Ravensberg et al. (157) found a significantly higher percentage of intact epithelium in smoking asthmatics than non-smoking asthmatics, and the authors concluded that the epithelial remodelling was similar to the COPD feature.

Changes in Reticular Basement Membrane (Rbm)

In asthma, subepithelial fibrosis mainly involves thickening of Rbm due to increased deposition of extracellular matrix (ECM) proteins, including collagens I and III, tenascin, and fibronectin. Fibroblasts are the main source of collagen I and II and are released in response to TGF-β. The fibroblasts sheath plays a crucial role in synthesizing and depositing these matrix proteins to seal the barrier, and the process involves hyperproliferative fibroblast and

the differentiation into myofibroblast. Brewster et al. (25) reported increased collagen thickness and myofibroblasts in asthmatic individuals and proposed that bronchial myofibroblasts responsible for the characteristic subepithelial fibrosis seen in allergic asthma. Further, increased deposition of ECM is associated with higher expression of submucosal MMP-9, a zinc-dependent endopeptidase capable of degrading collagen (86). Subepithelial fibrosis has been reported in different types of asthma, but degree of fibrosis did not provide the basis to differentiate from milder forms of asthma (37). Interestingly, fibrosis has also been reported in subjects with rhinitis but was found less marked than the asthmatics (32).

In COPD, findings on Rbm thickness have been conflicting (113, 124). Liesker et al. (124) found that the Rbm is thickened in both COPD and asthmatic patients compared to the normal; however, the Rbm is not significantly different between asthma and COPD. Interestingly, the Rbm composition in asthmatic and COPD was different, suggesting different types of airway remodelling changes and underlying airway inflammatory patterns in both diseases. Further, no significant correlation was noted between Rbm thickness or extracellular matrix components in either COPD or asthmatic patients and lung function.

On the contrary, Kosciuch et al. (113) reported a significantly low Rbm thickness in COPD patients compared to the asthmatics, with inflammation suggested as a critical factor impacting thickness. However, the study lacked a sufficient sample size to be conclusive. In addition, we have reported that the Rbm is quite fragmented in patients with COPD as part of the EMT process, which could explain the discrepancies in thickness measurements of the Rbm in the literature (182, 187, 189, 191, 193, 194).

Limited studies are available on the airway remodelling changes in ACO. A 3D-CT study (110) of ACO patient lungs found significant airway wall thickening and narrowing than COPD. These ACO patients had a history of variable respiratory symptoms and expiratory

441 airflow limitation (FEV_1% predicted >12% and FEV_1 of >200 mL BDR or four weeks of
442 anti-inflammatory treatment). Using the multidetector-row computed tomography, Niwa et al.
443 (142) found a thicker airway wall in ACO than the asthmatic patients, indicating a prominent
444 remodelling pattern than asthma. More recently, in asthmatic patients, a mild decrease in lung
445 elastic recoil has been seen. Interestingly, loss of lung elastic recoil is often observed in
446 COPD patients, and it was that the recurrent asthma attacks led to bronchiolar inflammation
447 with activation of a proinflammatory pathway with the activation of protease, cathepsin G
448 and MMP. All of these have a combined effect on the breakdown of parenchyma (66). A
449 recent endobronchial biopsy study by Papakonstantinou et al. (147) suggests that the thick
450 basement membrane in COPD could reveal ACO phenotype that might respond to
451 ICS/LABA; however, the study lacked statistical power and did not include the normal
452 healthy control. A similar result was found by Al-Kassimi et al. (4), demonstrating Rbm
453 thickening in 11 out of 14 non-emphysematous COPD patients displaying asthma features,
454 whereas, in comparison, emphysematous COPD patients had lesser thickened Rbm.

455 **Airway Smooth Muscle**

456 The potential roles of airway smooth muscle in the pathogenesis of asthma symptoms and
457 relation to airway hyperresponsiveness have previously been comprehensively reviewed (22,
458 96). Both hypertrophy and hyperplasia of ASM are seen in asthmatics, possibly due to
459 stimulation of ASM by growth factors such as platelet-derived growth factor or endothelin-1
460 released from inflammatory or epithelial cells (14). Further, ASM is capable of perpetuating
461 airway inflammation functions through biologically active chemokines and cytokines (e.g.,
462 TNF-α) as well as through cell adhesion molecules (CAMs) such as intercellular adhesion
463 molecule-1 and vascular cell adhesion molecule-1 (5). Additionally, an imbalance between
464 the expression of MMPs and MMP inhibitors TIMPs within the ASM could cause a

degenerative environment with increases in aberrant ECM deposition (8). Moreover, such an environment promotes aberrant fibrosis, leading to structural and mechanical abnormality (5).

COPD progression is strongly correlated to increased airway wall due to increased muscle layer apposing other morphological remodelling parameters (52). Increased muscle layer could contribute to airway responsiveness and negatively correlate with lung function in COPD (81). Although Hogg et al. (82), in their landmark paper, showed the increased smooth muscle in small airways of older COPD patients, other studies showed mixed results of either increase or no significant changes (24, 96, 201). Only mild smooth muscle hypertrophy was reported (59), whereas, in the small airway, we have recently reported significantly thickened smooth muscle in COPD current and ex-smoker patients compared to normal controls. Also noted was an increase in αSMA+ myofibroblasts in the SA wall of COPD patients related to pathological changes in the ECM scar proteins, collagen-1 and fibronectin (52).

Unlike asthma and COPD, the evidence on airway smooth muscle in ACO patient cohorts is again minimal. While Papakonstantinou et al. (147), reported no differences in airway smooth muscle cells in ACO patients compared to either asthma or COPD, Sha et al. (170), in a case report, suggested changes to the airway smooth muscle histology as a valuable but underutilized biomarker in disease phenotyping. They presented a case study of a 65-year-old woman who was initially diagnosed with COPD but following endobronchial biopsy histopathology, marked smooth muscle hypertrophy with thickened Rbm with squamous metaplasia was noted, and her treatment approach altered to advanced therapies for severe asthma. Thus, it highlighted the importance of smooth muscle histology for accurate phenotyping airway disease, especially with challenging ACO domains.

Vascular Changes

The vascular alteration in asthma includes increased angiogenesis and vessel numbers/size in the airway (22). Prominent features of fatal asthma are dilatation of blood vessels in bronchial mucosa, congestion, and oedema in the airway wall (47). The VEGF is an important controller of angiogenesis; presumably, it also increases the permeability of the blood vessels leading to dilation and oedema, which contributes to airway narrowing (35, 48, 85, 87). Angiogenesis has been reported in mild asthma and severe corticosteroid-dependent asthma (98).

The main vascular remodelling changes in COPD involve increased pulmonary muscular arterial intimal thickness due to proliferating longitudinally oriented smooth muscle cells without significant any differences in cellular and extracellular matrix components (elastin, collagen, proteoglycans) and increased medial thickening, leading to reduction of arterial luminal diameter (167, 168). The other changes include hypo-vascular lamina propria and hyper-vascular Rbm in the large airways of smokers and COPD, as we previously reported (182, 194, 195, 197). In addition, Reimann et al. (158) reported an enhanced S100A4 expression in remodelled pulmonary arteries of COPD patients, which are upregulated in fibrosis (181). Therefore, higher S100A4 expression in the pulmonary vasculature of COPD patients indicates that the process of the endothelium to mesenchymal transition may be active and may have a possible role in vascular remodelling and fibrosis in COPD. The evidence of vascular remodelling in ACO is still evolving.

Epithelial to Mesenchymal Transition

Epithelial to mesenchymal transition (EMT) is when epithelial cells lose their epithelial functionality and characteristics, i.e., cell-cell adhesion and apico-basal polarity, and attains a mesenchymal phenotype that includes migration, invasion, and increase in ECM components.

EMT has been classified into 3 types (103). The Type I EMT occurs at the early development stage; the Type II EMT leads to organ fibrosis and obliteration; and the Type III EMT can induce the formation of pre-malignant stroma when associated with angiogenesis (108, 186). When active, the Type II EMT is devoid of angiogenesis and is the distinguishing compared to Type III EMT, which is angiogenesis prominent. Some in-vitro studies suggest an increase in EMT activity with induction lead by either TGF-β or EGF in epithelial cells derived from asthmatics and normal subjects (42, 72). Although micrographs of endobronchial biopsy of asthmatic children shown in a case series indicated the possibility of EMT, further in vivo or patient studies are warranted to verify whether EMT is active process asthma (101, 191).

We have previously shown that EMT is an active process in the large airways of smokers and patients with COPD, as indicated by reticular basement membrane (Rbm) fragmentation and hypercellularity (187). The Rbm fragmentation is the key structural tissue hallmark of active EMT, facilitating the epithelial transition and migration into the underlying lamina propria. We have reported clefts within the Rbm with cells expressing MMP-9 and the early fibroblast transition marker, S100A4, also in the basal epithelium and epithelial activation marker EGFR, all suggesting active EMT. Important pathological implications of EMT in COPD are fibrosis and obliteration of small airways and lung cancer (50, 52, 58, 125, 130, 179, 180). The key cell population in fibrosis is myofibroblasts, which could be derived from several sources, including circulating fibrocytes, epithelium, endothelial cells, pericytes and resident lung stromal cells(79). As we published previously, Type III EMT is active in smokers and patients with COPD, responsible for malignant transformation in these patients, leading to lung cancer (128, 130). In addition, EMT can significantly change the airway wall ECM characteristics, making the airway more vulnerable to compression and obstruction expiratory dynamics. Therefore, the role of active Type II EMT in airway fibrosis or obliteration cannot be ruled out. Indeed, Milara et al. (135) reported that the EMT process in

the small bronchi among smokers and COPD patients could potentially contribute to the small airway wall thickening.

EMT in COPD is potentially driven by the "canonical" TGFβ pathways via the phosphorylated (p) SMAD transcription factor fingerprint as indicated with higher TGFβ expression the airway wall of COPD patients, high expression of *pSMAD2/3* and a reduced *pSMAD 7* expression which is positively associated to airflow obstruction (127). We also reported that vessel associated TGFβ increases in the Rbm, which may have implications for driving both Type II and III EMT in smokers and patients with COPD (195). Furthermore, the transcription factor clusters of β-catenin/Snail1/Twist, which implicates EMT, were upregulated in COPD and associated with airflow obstruction (129). Therefore, blocking EMT in COPD possibly brings substantial therapeutic potential. Previously, we have reported the suppression of EMT-related changes in large airways of COPD patients treated with fluticasone propionate in a randomized controlled study. We found a reduction in Rbm fragmentation, EGF receptor, basal epithelial and RbmS100A4, and MMP-9 expression in the treatment group compared to placebo (188). However, we observed that vascular changes related to the Rbm did not go away after the treatment, suggesting more extended treatment requirements for hypervascularity (196). Taken together, we believe that EMT is a critical process in COPD, contributing towards airway fibrosis and lung cancer and could have an essential role in ACO but this warrants further work. So far, no studies have been conducted to identify EMT in ACO; however, given increasing evidence of its prominent role in COPD, it would be natural to presume that EMT as a phenomenon is crucial to ACO pathogenesis.

3.0 Treatment options for ACO

Undoubtedly, the management of asthma and COPD is currently at an advanced stage, with several treatment strategies currently available to control disease progression (2, 3, 9, 57,

185). The mainstream asthma pharmacotherapy includes treatment with short-acting β2 agonist (SABA) (e.g., albuterol), LABA (e.g., formoterol, salmeterol, and vilanterol), ICS along with anticholinergics, oral corticoids (OCs) (e.g., prednisone), and anti-inflammatory biologics (Table 2). Constant bronchodilation continues to be the major objective of COPD management that includes treatment with SABA (salbutamol, terbutaline, and fenoterol) and LABA (salmeterol, formoterol, and indacaterol). Treatment with short-acting (ipratropium and oxitropium) and long-acting muscarinic receptor antagonists (LAMA) (tiotropium) is also effective on symptoms, airflow limitation, and exacerbations (3). The GOLD document does not recommend long term monotherapy with ICS as well as OCs. Adding up LAMA with LABA/ICS therapy is beneficial in reducing the exacerbation and improving the lung functions.

There is no firm therapeutic approach available for ACO treatment due to the lack of evidence in this patient population, making it difficult for clinicians to make certain informed treatment recommendations. Clinicians' treatment decisions are based on the more prominent phenotype, i.e., asthma or COPD like features, that may or may not be present in the ACO patients (46, 200). The GOLD 2020 advised people with ACO to follow recommendations for asthma therapeutic approaches. In all patients with chronic airways diseases, advice focuses on smoking cessation, ensuring appropriate inhaler technique, optimising adherence to therapy, identifying and avoiding the risk factors, appropriate treatment for comorbidities, utilizing pulmonary rehabilitation, and vaccinations (207). The GINA 2020 mentions using low or medium-dose ICS as the initial treatment but reminds prescribers about the potential occurrence of adverse events such as pneumonia and considering the add-on treatment with LABA and LAMA for managing the COPD features (2, 54, 55, 183, 184). The LAMA/ICS/LABA triple combination, once-daily treatment, has shown significant improvements in lung function from the baseline in ACO patients compared to ICS/LABA

585 dual therapy (90). Treatment with ICS+LABA combination once daily was also demonstrated
586 a substantial improvement in lung functions in ACO patients with episodic respiratory
587 symptoms, increased airway variability (AHR or BDR), and incompletely reversible
588 obstruction in the airway (91). Very limited data are available for the appropriate first-line
589 therapy for ACO patients. Treatment with ICS is considered for ACO patients, and the
590 available literature does indicate that the ICS response is related to the prognostic values in
591 asthma and COPD. However, no evidence reflects the ICS response in ACO patients when
592 defined by the reversible FEV_1.

593 Monoclonal antibodies (anti-IgE, anti-IL-5, anti-IL-5ra, and anti-IL-4) targeting the patients
594 with prevalent Th2 inflammation could be promising for ACO. In a post hoc, exploratory
595 efficacy analysis of omalizumab in the ACO (asthma patients with COPD diagnosis or self-
596 reported, n=56) and non-ACO (asthma patients, n=681) patients, the improvement in the
597 asthma outcomes were noted in both ACO and non-ACO patients. Of note, no significant
598 differences in the baseline demographic profiles of these groups were noted (74). Further, the
599 study of mepolizumab, an anti-IL-5 (Table 2), in patients with COPD eosinophilic phenotype
600 was found to reduce the eosinophil counts and annual exacerbation rate (150). It is to be
601 noted that the study lacked asthma and ACO patients; however, COPD patients were
602 stratified according to blood eosinophil counts ($\geq 150/mm^3$ at screening or $\geq 300/mm^3$ at any
603 time during the previous year), that is a Th2 inflammatory marker. Although trial with
604 benralizumab in COPD patients with eosinophil counts of less than and greater than $220/mm^3$
605 and frequent exacerbation showed substantial blood and sputum eosinophils, they did not
606 correspond to the substantial decrease of exacerbation rates. Interestingly, the trial also
607 excluded asthmatic patients and ACO patients (40). Therefore, the responder may be
608 identified Th2 phenotypes, and it is possible that these therapies could benefit individuals

609 with ACO. Oher potential monoclonal antibodies for ACO treatment are reslizumab and
610 dupilumab; however, the evidence on efficacy in ACO is still evolving.

611 The PDE4 inhibitors such as roflumilast could be a potential treatment option for ACO
612 patients as an alternative to the bronchodilator and ICS. Roflumilast is an approved drug for
613 treating COPD but not asthma, although a recent metanalysis of the major database found
614 that roflumilast (500 μg) significantly improved FEV_1, peak expiratory flow, asthma control
615 and exacerbations (126).

616 Macrolides are helpful to treat COPD patients because of their anti-inflammatory,
617 immunomodulatory, and antibiotic effects (149); however, there is a lack of evidence of their
618 effectiveness in asthma patients. A Cochrane review reported that the macrolides in
619 managing chronic asthma were no better than the placebo (111). Interestingly, recent
620 evidence of long-term treatment with erythromycin in ACO patients found reduced airway
621 inflammation, total cells, neutrophils, and neutrophil ratio in induced sputum in addition to
622 the significant reduction of exacerbations (141). Hence, macrolides could be an effective
623 option for treatment of ACO patients with neutrophilic inflammation.

624 Wu et al. (212) recently found a protective association between metformin and decreasing
625 respiratory exacerbation rate in ACO patients (n=510), defined as simultaneous physician-
626 diagnosed asthma before 40 years of age, and COPD from the Genetic Epidemiology of
627 COPD study cohort. However, randomized clinical trials are required to verify these findings
628 and warrant detailed prospective investigations.

629 ## 4.0 Conclusion

630 In this review, asthma and COPD related research from the last decade have been surveyed.
631 Notably, we have summarized the pathological aspects of ACO, asthma and COPD,
632 emphasizing the role of both innate and adaptive immunity, and presented the importance of

airway remodelling in individual disease phenotype. The interplay of immune systems and airway wall changes seems to be important but remains understudied in these chronic airway diseases. It is urgent to format a consensus that can better explain ACO pathological characteristics as a distinct disease phenotype. Most ACO research conducted is either systemic or sputum based, and there is a poor prognosis on the specific site-related inflammation and remodelling changes. Discovering the pathobiology of ACO would provide further understanding and help to identify diagnostic criteria, allowing clinicians to identify these select patient populations, thus providing better therapeutic interventions.

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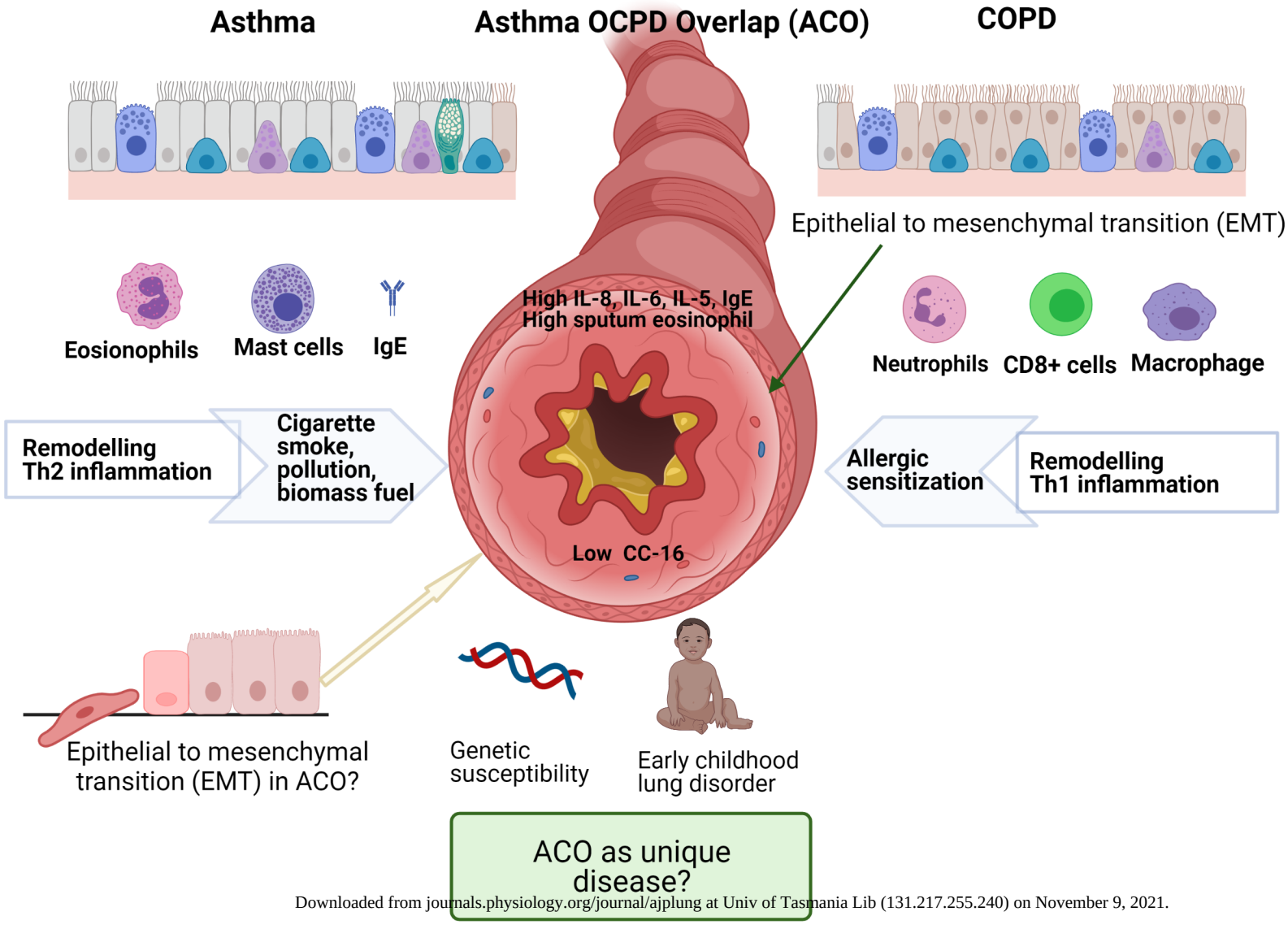
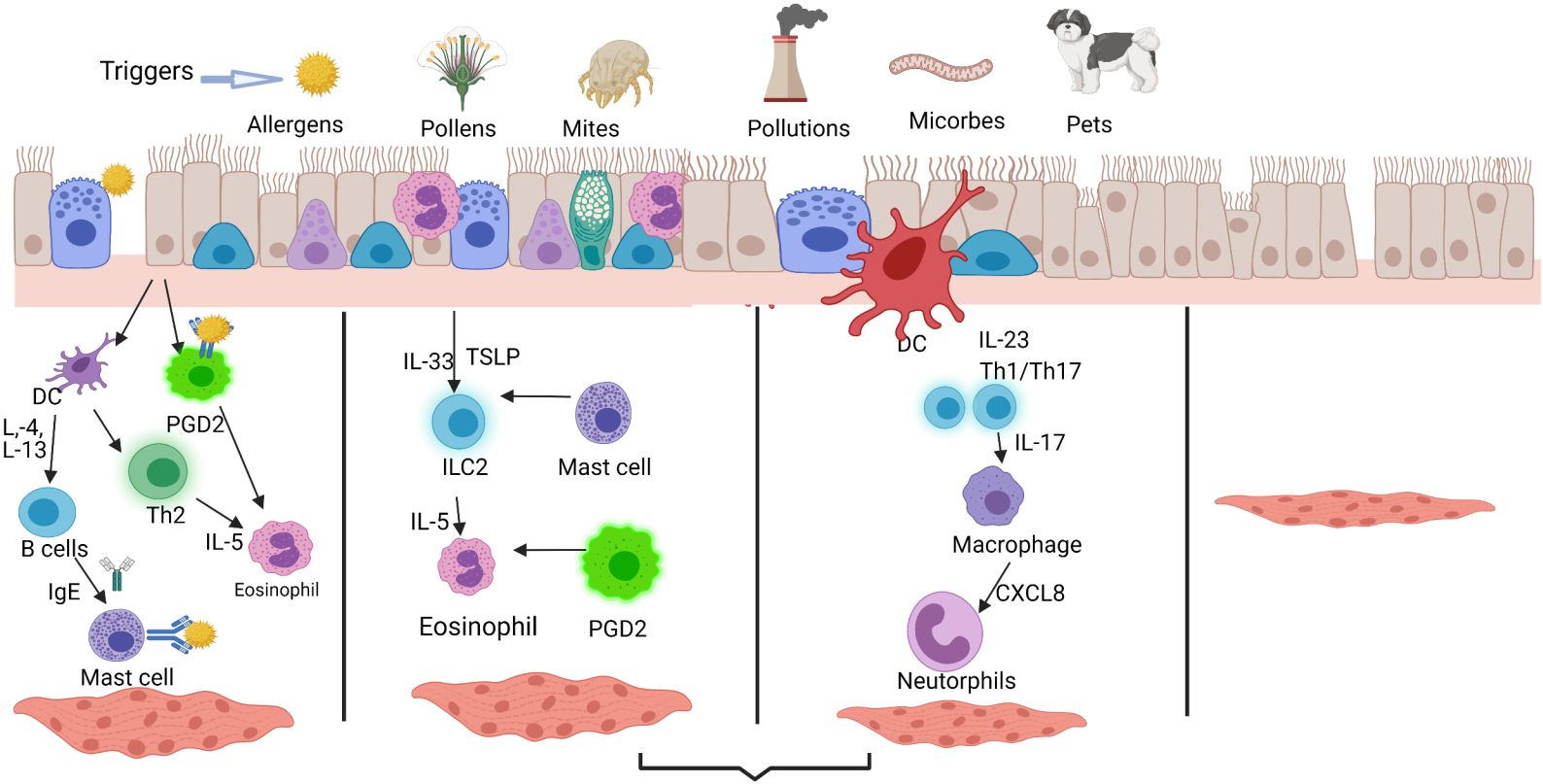
Figure 1 Pathological Features of Asthma COPD Overlap: Possible mechanisms for the asthma-COPD overlap (ACO). According to the Dutch hypothesis (which states the shared origin of asthma and COPD) if asthmatic patients are exposed to toxic inhalants such as cigarette smoke or biomass fuel, that causes COPD, may develop ACO. Similarly, COPD patients may develop asthma like features when the patients are sensitized with allergens. Inversely, the British hypothesis states that both the asthma and COPD are distinctively unique diseases. Impaired lung function could have an early origin. Progression from prenatal insult to paediatric disease and finally to obstructive airway disease in adulthood may have a complex interaction between genetics and epigenetics. Thus, early childhood events such as impaired lung functions may lead to ACO and further, the genetic susceptibility e.g., SNP in CSMD1 demonstrated implications for ACO. Abbreviations: IL: Interleukin; CC-16: Club cell secretory protein. Created with BioRender.com

Figure 2 Immunopathological Features of Asthma: Abbreviations: CXCL8=C-X-C motif chemokine ligand 8, IL: interleukin, ILC2: type 2 innate lymphoid cell, ILC2=type 3 innate lymphoid cells, PDG2: prostaglandins D2, Th: T helper, TSLP: thymic stromal lymphoprotein. √√ represents most common, √ represent presence, and X represents absence. Created with BioRender.com.

Figure 3 Inflammatory Cascade and Features of COPD: Adapted from Sapey et al. (169) CXCL=C-X-C motif chemokine ligand, IL: interleukin, ILC2: type 2 innate lymphoid cell, Th: T helper, The precipitating event such as bacterial or cigarette smoke or an environmental trigger causes inflammation of the airway epithelium, subsequently activating the resident immune cells including macrophages and T cells leading recruitment of neutrophils, but also T cells, B-cells and eosinophils in the lung tissue, following chemokines released by epithelial, endothelial, and resident immune cells. Macrophages and epithelial cells (EMT) release growth factors that activate fibroblasts. Recruited immune cells secret cytotoxic granular contents, ROS and proteinases into the tissue and these events generally associated with the development of mucus secretion, emphysema, and small airways remodelling thus progression of COPD.





Eosiniphilic asthma (Th2/allergic inflammation)
Eosinophil (√√)
Damaged epithelium (√√)
Thickned RBM (√√)
ASM (√√)
Mucous (√)
Neutrophils (x)

Eosinophilc asthma (non-allergic inflammation)
Eosinophil (√√)
Damaged epithelium (√√)
Thickned RBM (√√)
ASM (√√)
Mucous (√)
Neutrophils (x)

Mixed glanulocytic asthma
Damaged epithelium (√√)
Mucous (√√)
Thickned RBM (√)
ASM (√)
Eosinophil (√)
Neutrophils (√)

Neutrorphilic asthma
Neutrophils (√√)
Damaged epithelial (√√)
Mucous (√√)
Thickned RBM (√)
ASM √)
Eosinophil (x)

Paucigranulocytic asthma
Damaged epitheliuml (√)
ASM (√)
Mucous (√ or x)
Thickned RBM (√ or x)
Neutrophils (x)
Eosinophil (x)

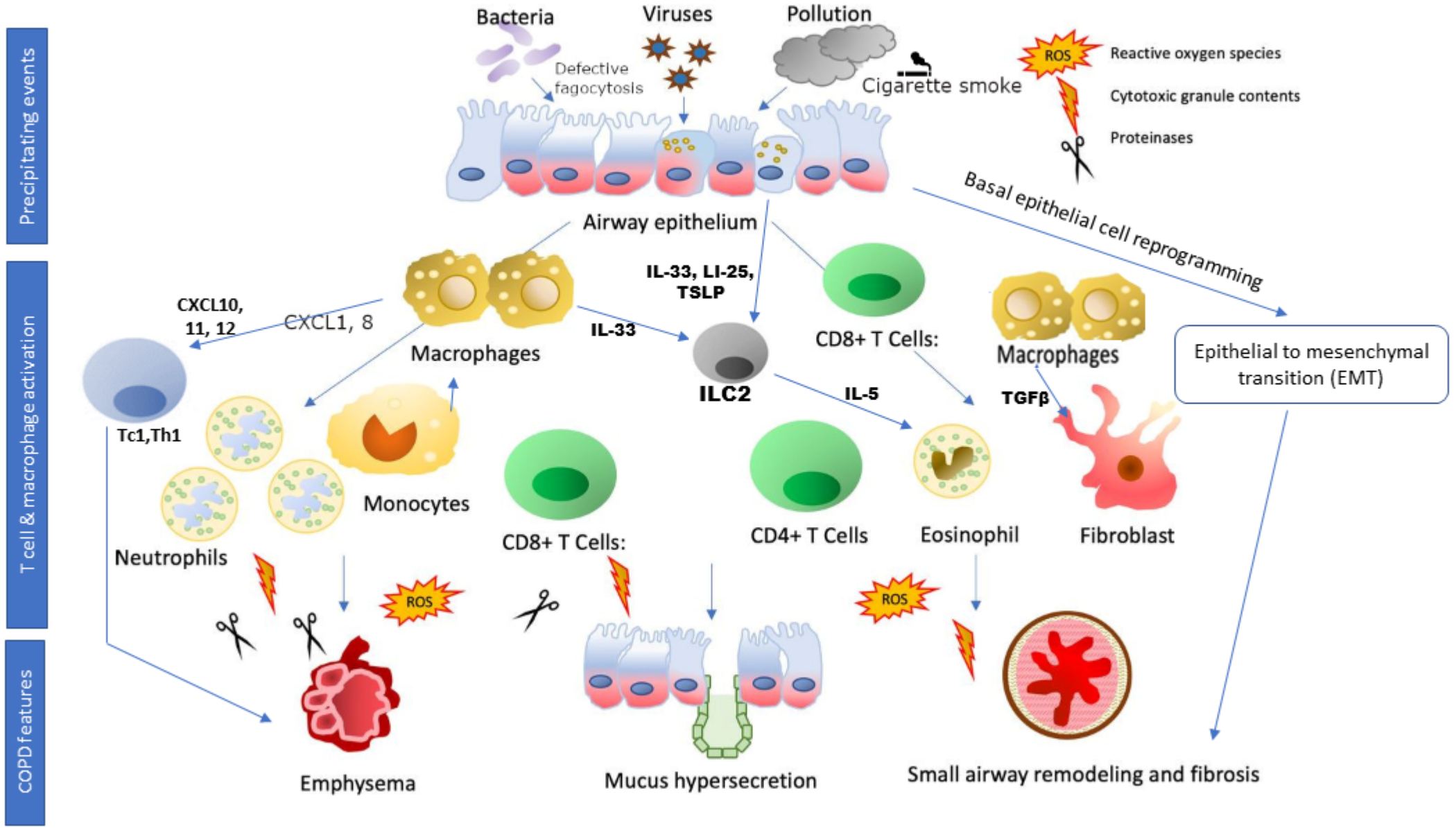


Table 1 Comprehensive summary of inflammatory cells including innate and adaptive immune cell in asthma, COPD and ACO.

Disease type/references	Cell type	Sample type/ technique used	Subjects	Findings
Asthma				
(119).	CD4+ and CD8+ lymphocytes	Induced sputum and whole blood/ flowcytometry	NC (nonatopic) 6, COPD CS 7, Asthma NS 8	Sputum T lymphocytes are predominant phenotype (CD103+ CD69+) with normal numbers of CD4+ and CD8+ T cell populations.
(173)	IL-17A, IL-8, IL-6, neutrophils, and eosinophils	Endobronchial biopsy/IHC, ELISA	Asthma CS 8, Asthma NS 8, and Asthma ES 2	Significant elevation of neutrophil, IL17A, IL6 and IL8 in bronchial mucosa of asthmatic smokers as compared to no-smokers.
(23)	CD8, CD4, NK cells, dendritic cells (DC), Myeloid derived suppressor cells (MDSC), regulatory T cell	BAL/ flowcytometry	Asthma (atopic) 24	Elevated expression of CD30 on Treg; increased eosinophil but no significant increase in IL-4; significantly high CD4+ T lymphocytes while a significantly low in CD8+ T lymphocytes; significantly high plasmacytoid dendritic cells (pDC) after allergen challenge
(213)	CD8, CD4, and CD127	Blood, sputum, bronchial biopsies, and BAL/ Fluorescence-activated cell sorting	Healthy 37, eosinophilic severe asthmatic 34, non-eosinophilic severe asthmatic 54	CD8+ is markedly increased in eosinophilic patients both in blood and airway. Airway CD4+ cells and blood CD127+were not significantly increased.
(10)	CD45+, CD3+, CD4+, CD8+, mast cells, eosinophils, neutrophils, CD25+ (IL2)	Mucosal biopsies/IHC	NC (nonatopic) 9, Asthma (atopic) 11, healthy 10	Increased CD45, CD3, and CD4- and CD8-positive cells, eosinophils, CD+ cells in asthmatics. No significant mast cells, neutrophils, or Leu-M3 + cells in the airway mucosa of asthmatics.
(73)	Macrophage, eosinophil, neutrophilCD8+CD28−, CD8+CD56+, perforin, INF-γ	Sputum/IHC, Flowcytometry	NC 10, severe asthmatic, mild asthmatic 12	Significant elevation of eosinophils and lymphocytes in severe asthmatics. Increased CD8+CD28−, CD8+CD56+ cells in severe asthmatics, compared to healthy subjects and mild asthmatics with parallel decrease of the CD8+CD28+. CD8+CD28− cells produced high perforin and low INF- γ in sever asthmatic patients.
(6)	Eosinophils, neutrophils, mast cells, CD3+, CD4+, CD8+, and CD25+ (IL2)	Bronchial biopsy tissue/IHC	NC 7, asthma (atopic 13; non atopic 9) 21	In atopic asthma patients had elevated eosinophils, mast cells, and T lymphocytes (CD4+), whereas nonatopic asthmatics mainly showed high neutrophils and mast cells.
COPD				
(45)	DC	Resected lung tissue/IHC Sputum/	Never smoker 10, smoker without COPD 9, smoker COPD	Significantly high DC number in the epithelium and adventitia of small airways in COPD compared with never-smokers and smokers without COPD

Disease type/references	Cell type	Sample type/ technique used	Subjects	Findings
		Flowcytometry	(GOLD I 10, GOLD II 16, GOLD III-IV 10)	
(7)	Mast cells	Resected tissue/ IHC	NC 8, smokers 7, COPD-CS 5, COPD-ES 20	The density of connective tissue mast cells in patients with very severe COPD was significantly higher than in controls.
(163)	Neutrophils, eosinophils, mast cells, macrophages, CD4+ and CD8+	Lung tissue, pulmonary arteries/ IHC	NS 8, NLFS 6, smoker COPD	Smokers with COPD have an increased number of CD8+ cells. Arteries infiltrated by neutrophils, eosinophils, mast cells, macrophages, and CD4+ cells were not significantly different in examined groups.
(51)	Macrophage (M1/M2)	Resected tissue and BAL/ IHC and ELISA	NC (BAL 11, SA 10), NLFS (BAL 13, SA 11), COPD CS (BAL 16, SA 9), COPD ES (BAL 13, SA 11)	Decrease in M2 and increase in M1 macrophages in the small airway in COPD and NLFS compared with normal with a reciprocal decrease in M2 macrophages
(156)	Neutrophil	Biopsy tissue/ IHC, In Situ Hybridization	Stable COPD 7 (smokers), exacerbated COPD 15 (smokers), NC 15	Both stable and exacerbated COPD had significant increase in neutrophils with an association with upregulation of both CXCL5 and CXCL8.
(70)	B Cells (CD20+)	Bronchial biopsies from large airway/ IHC	COPD 114 (CS 72, ES 42), NC (CS 27. ES 1)	High B-cell numbers in patients with COPD as compared to controls and higher in patients with GOLD severity stage 3 than stage 2
(41)	CD4+ and CD8+ T cells, macrophage, (CD80+ CD163- and CD80+ CD163+), monocytes	Fresh lung tissue and venous blood/ flowcytometry, Transcriptomic analysis	NS 12, smokers 9, COPD ES 16, COPD CE 28	COPD CS had significant reduction in the proportion of T-cells that involved both CD4+ and CD8+ T cells; increase in the proportion of macrophages (CD80 + CD163+ and CD80 + CD163-) lung monocytes distributed differently between the study groups due to an increase of monocytes in COPD-CS.
(43)	ILC1, ILC2, NCR+ ILC3 and NCRILC3 (CD45+); CD45+ LinCD127+	Resected tissue/ flowcytometry	NC 5 (NS 3, CS 2), COPD 11 (CS 5, ES 6)	High frequency of NCR+ILC3 with increase in IL-17A and IL-22 expressing ILC in COPD compared with controls, whereas IFN-γ and IL-5 expressing ILC were similar in control and COPD.
(33)	CD4 and CD8 T cells	Biopsy tissue/I HC in large airways	NC 15 (ES 3), COPD CS 7, COPD 20 ES	Increased expression of CD4 and CD8 cells expressing IL-17 cytokines.
(62)	CD8+ and CD4+ co-expressing TLR	Lung resected tissue/ Flow cytometry	NLFS 14, COPD (ES 15, CS 5)	Increase in CD8+ T cells expressing TLR1, TLR2, TLR4, TLR6 and TLR2/1 over smokers without COPD.
(164)	neutrophils, macrophages, CD4+ and CD8+ T-cells	Resected tissue/ IHC	NC 7, COPD 9 (with smoking history)	Increased number of CD8+ T-lymphocytes

Disease type/references	Cell type	Sample type/ technique used	Subjects	Findings
(165)	Macrophages, neutrophils, CD45+ cells, CD4+ and CD8+ cells	Resected tissue/ IHC	NS 9, NLFS 6, COPD Smoker 10	Significant increase in CD45 cells, macrophages, and CD8+ cells in COPD patients as compared to non-smokers
(53)	neutrophils, CD68+ and CD8+ cells	Small and large airway biopsy tissue/ IHC	NC 35, NLFS 31, COPD-CS 21, COPD ES 24	Decreased neutrophil, macrophage in large airway and a significant increase of CD8+ cells in small airway.
Asthma COPD Overlap (ACO)				
(12)	IL-4, IL-5, IL-9, IL-13, IL-1b, IL-6, and TNF-α	Sputum, Serum/ ELISA and flowcytometry	Asthma 23, COPD 28, ACO 24	Both serum and sputum, IL-4, IL-5, IL-9, and IL-13 in COPD as compared to asthmatic and TNF-a, IL-1b, IL-6 in bronchial asthmatic as compared to COPD were highest. lowest CD4: CD8 ratio was found in the bronchial asthma patient group and the highest ratio was found in the COPD patient group.
(107)	CD3+, CD4+, CD8+, CD4+/CD8+, CD19+, CD16+56+, IgE, TNF-α, IL-4, and IFN-γ,	Serum/ ELISA, flowcytometry	Control 20, COPD 44, Asthma 39, ACO 12	Increase in CD3+CD8+ lymphocytes, B lymphocytes, LTB4 in ACO patients as compared to control, asthmatic, and COPD alone.
(63)	Eosinophil, neutrophil, IL-6, NGAL, YKL-40, IL-13, MPO	Sputum/ ELISA	NS 14, Healthy-CS 14, Asthma-ES 9, -CS 6, COPD-ES 7, -ES 12, ACO-ES 11, -CS-7	IL-13 and MPO were higher ACO patients compared to healthy and discovery cohort. NGAL, IL-6, and YKL-40 were elevated in ACO as compared to NS.
(64)	Eosinophil, neutrophil, lymphocyte, macrophage	Sputum/ staining and microscopic	Asthma 142, COPD 160, ACO 72, all smokers	Higher neutrophils in COPD, higher eosinophils in asthma and ACO, eosinophilic difference in asthma and COPD are not apparent, elevated macrophage in asthma compared to ACO
(147)	Lymphocyte, eosinophil, granulocytes	Endobronchial biopsy/ stain	COPD 129 (CS-47), Asthma-19, COPD with asthma 18 (CS 16)	No difference in lymphocytes, eosinophils, or granulocytes infiltration in tissue among the COPD patients with or without asthma.

Abbreviations: BAL: Bronchoalveolar lavage; CD-cluster of differentiation; CS- Current Smokers; COPD: Chronic obstructive pulmonary disease; DC-Dendritic cell; ELISA: Enzyme-linked immunosorbent assay; ES- Ex Smokers; IHC- Immunohistochemistry; IL-Interleukin; ILC: Innate lymphoid cells; LTB4-Leukotriene B4; NCR: Natural cytotoxicity receptor; NC- Normal Control; NGAL-Neutrophil Gelatinase-associated Lipocalin; NLFS- Normal Lung Function Smokers; PB- Peripheral blood; PMO-myeloperoxidase; SA: Small airway; TLR-Toll-like receptor family; TNF-α-Tumour Necrosis Factor alpha; UA- Unavailable; YKL-40: Chihtinase-3-like protein

Table 2 Representative Therapies in Use/under Investigation for Asthma, COPD and in overlap.

Reference	Treatments	Target	Patient population	Outcomes
(146)	Mepolizumab, injection for subcutaneous use	anti–interleukin-5	Severe asthmatics with at least 2 exacerbations previous year and treated with glucocorticoids; peripheral BEC ≥150 cells/μl at screening or 300 cells/μl in the previous year	Significant reduction is exacerbation as compared to placebo and increase in FEV_1 values as compared to baseline. Similar safety profile as seen in placebo
(30)	Reslizumab injection, for intravenous use	anti–interleukin-5	Asthmatics inadequately controlled by medium-to-high ICS; BEC 400 cells/μL or higher and one or more exacerbations in the previous year.	Significant reduction in the asthma exacerbation rate. Common adverse events on reslizumab were similar to placebo.
(139)	Benralizumab injection, for subcutaneous use	interleukin-5 receptor alpha-	Severe asthmatics with BEC ≥150/mm^3	Significant decrease exacerbation/year, improved symptom controls
(29)	Dupilumab, injection for subcutaneous use	interleukin-4 and 13	Sever asthmatics above 12 years old with current treatment with medium-to-high ICS + up to two additional controllers; BEC <300 or ≥300/ mm^3	Significantly lowers rates of exacerbation as compared to placebo; better lung function and asthma control. Greater benefit in patients with high baseline eosinophil. The safety profile was comparable with placebo
(136)	Omalizumab/ rhuMAb-E25 injection, for subcutaneous use	immunoglobulins E (IgE)	Moderate or severe allergic asthma	Improvements in daily asthma symptom score. No significant difference in adverse events profile comparing the placebo.
(38)	Tezepelumab*, subcutaneous injection	TSLP	Severe Uncontrolled adult Asthmatics treated with LABA and medium-to-high ICS dose	Significant reduction of asthma exacerbation rate, that occurred irrespective of baseline BEC
(84)	Etanercept*	TNF-α	moderate-to-severe persistent asthmatics	No statistically significant clinical efficacy between the treatment and placebo group, however the adverse event profile remains similar to that of placebo.
(27)	Imatinib, 200 mg/day 2 weeks and then 400 mg/day, oral	KIT proto-oncogene receptor tyrosine kinase and mast cells	Severe, refractory asthmatic uncontrolled with inhaled beclomethasone, and at least one additional controller medication, score on Asthma Control Questionnaire (ACQ-6) of at least 1.5.	Imatinib reduced AHR, mast-cell, and tryptase release. Muscle cramps and hypophosphatemia were more common in the imatinib group than in the placebo group
(140)	CSJ117*, an Anti-TSLP mAb fragment (46 kDa)	TSLP	-	-
(121)	Montelukast, 10 mg tablets; oral	cys-LT receptor (LRA) antagonist	Non-smokers (15-45 years) asthmatics for more than one year using inhaled β2 agonist with decreased FEV_1 after methacholine challenge	Significant protection in QD treatment against exercise induced asthma over a 12-week period with less required β2 agonist.

			and exercise challenge	No significant differences between treatment and placebo group in the frequency of clinical or laboratory adverse effects.
(150)	Mepolizumab 100 mg and 100 mg Subcutaneous injection	anti–interleukin-5	COPD for at least 1 year; history of moderate or severe exacerbations when taking ICS-based triple maintenance therapy; BEC ≥300/ mm^3 and ≥150/ mm^3 at screening	Mepolizumab (100 mg) had reduction on the annual rate of moderate or severe exacerbations among patients with higher BEC at screening as compared to placebo. Safety profile was similar to that of placebo.
(28)	Roflumilast 500 μg once a day, oral	Phosphodiesterase-4 (PDE4) inhibitor	COPD >40 years old, with severe airflow limitation; chronic cough and sputum production.	In roflumilast significant increase of prebronchodilator FEV_1, moderate to severe exacerbation rate reduced significantly.in a subset of COPD patients. Class-related adverse effects that usually arise soon after initiation of treatment.
(177)	CHF6001*/ inhale route	novel PDE4 inhibitor	COPD patients with post-bronchodilator FEV_1/FVC ratio < 0.70 and FEV_1 ≥ 30% and ≤ 70% predicted, CAT score ≥ 10, and a history of chronic bronchitis, receiving triple inhaled therapy	Reduction of sputum biomarkers such as leukotriene B4, CXCL8, MIP-1β, MMP9, and TNFα also significantly decreased SP-D levels in the blood. Reduced number of PDE4 class related adverse events.

* Under investigation.

Abbreviations: AHR: Airway hyperresponsiveness; BEC: Blood eosinophil count; CAT: COPD Assessment Test; COPD: Chronic obstructive pulmonary disease; CXCL8: C-X-C motif chemokine ligand 8; FEV_1: Forced expiratory volume in 1 second; FVC: forced vital capacity; ICS: Inhaled corticosteroids; MIP: Macrophage inflammatory protein; MMP: Matrix metallopeptidase; SP-D: Serum surfactant protein; TNF: Tumor necrosis factor; TSLP: Thymic stromal lymphopoietin.