



Structure and function of small airways in asthma patients revisited

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Small in size, big in impact: structural and functional alterations of the epithelium, extracellular matrix and airway smooth muscle are present in the small airways of asthma patients. These alterations may play a pivotal role in asthma pathophysiology. <https://bit.ly/3IIT62>

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ABSTRACT Small airways (<2 mm in diameter) are probably involved across almost all asthma severities and they show proportionally more structural and functional abnormalities with increasing asthma severity. The structural and functional alterations of the epithelium, extracellular matrix and airway smooth muscle in small airways of people with asthma have been described over many years using *in vitro* studies, animal models or imaging and modelling methods. The purpose of this review was to provide an overview of these observations and to outline several potential pathophysiological mechanisms regarding the role of small airways in asthma.

Introduction

In 1968, HOGG *et al.* [1] were the first to describe that in the lungs of patients with COPD the resistance in the smaller bronchi and bronchioles (2–3 mm in diameter) was significantly increased up to 40 times compared with normal lungs. Resistance was measured by using the retrograde catheter technique. With this technique the catheter is extended through the parenchyma and the bronchial wall, instead of extending the catheter into the peripheral airways *via* the trachea. This technique made it possible to differentiate between large and small airway resistance. It was used earlier by MACKLEM and MEAD [2] in healthy canine lungs, where they observed that in healthy lungs the small airways normally contributed only 10% to total airway resistance. However, HOGG *et al.* [1] found that besides an increase in resistance, there were extensive morphological changes in these small airways. Based on these findings they introduced the term “small airways disease” (SAD). In current literature the term “small airways” is used for airways with an internal diameter ≤ 2 mm or internal perimeter of ≤ 6 mm. The number of airway generations needed to reach the small airways through the bronchial tree starting in the trachea can vary from four to 14 generations, with the majority being reached in seven to eight generations [3, 4].

In asthma, the larger airways were most frequently studied because of prominent changes observed in them at autopsy and ease of access for biopsy. Therefore, at first, asthma has been considered as a condition with important involvement of the large airways. However, over the years, techniques to assess the small airways have improved such that the role of the small airways in asthma pathophysiology has

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come into focus. A systematic review showed that in 50–60% of people with asthma, SAD is present [5]. In a large prospective study in adults (ATLANTIS study) it was shown that the small airways are involved across almost all asthma severities, and that they show proportionally more structural and functional abnormalities with increasing clinical severity of asthma [6]. To date, it is unclear whether the involvement of the small airways is related to severity of asthma or to a specific group of patients with asthma [7].

Since the introduction of the term SAD by HOGG *et al.* [1], several hundred publications about the small airways have appeared in the medical literature, describing structure, function, diagnostic measures and therapeutic options. However, only a few studies have reviewed the potential mechanisms and associated pathophysiological changes leading to SAD in patients with asthma. Therefore, we aim to discuss the structural and functional changes of the small airways of humans in asthma in detail.

For this review we conducted an extensive literature search by using PubMed. We used the following terms: “structure”, “function”, “remodelling”, “asthma”, “small airways”, “peripheral airways”, “distal airways”, “airway smooth muscle”, “extra cellular matrix”, “epithelium”, “imaging”, “ventilation defects”, “mechanics” and “(patho)physiology” in different combinations, orders and in alternate spelling. From this literature search we retrieved approximately 1700 papers. On the basis of relevance for the aim of our review, approximately 150 papers were selected and reviewed. In the first section of the review we discuss the structural components in relation to SAD, followed by what is currently known about functional issues in relation to SAD and finally, we discuss the role that diagnostic measurements, imaging and modelling can play in our understanding of SAD. Inflammatory cells and pathways are of key importance in the pathophysiology of asthma. However, due to the extent and complexity of these cellular mechanisms and pathways, we have not included them as they are beyond the scope of this review. We suggest the in-depth review by LAMBRECHT *et al.* [8] for more information on inflammatory cells and pathways in asthma.

Epithelium

In healthy airways the epithelium lines the airways as a continuous sheet (figure 1). In the large airways the epithelium is pseudo-stratified and becomes columnar and cuboidal in the small airways. Furthermore, the epithelium consists of goblet, serous, basal, club and immune cells [9]. Compared with the large airways, the epithelium of the small airways has a greater proportion of ciliated and club cells and few or almost no goblet cells (figure 2) [10]. The epithelial cells are held together by apical tight junctions, basal adherens junctions and desmosomes [11].

The predominant function of the epithelium was long thought to be a physical barrier to pathogens. The production of mucus, the mucociliary escalator, cell-to-cell adhesion proteins and inflammatory mediators are all components of this barrier function. Mucin protects the airway epithelium from inhaled pathogens by trapping them and preventing them from penetrating the mucus into the epithelium. Moreover, mucins play a vital role in clearance of pathogens, pollutant particles and inflammatory stimuli from the airways [12]. MUC5B and MUC5AC are the main gel-forming mucins in human airways. MUC5B is secreted throughout the airways in healthy small and large airways. In healthy lungs almost no MUC5AC is produced in the small airways [13]. In addition, mucins in combination with the periciliary layer and ciliated epithelium form the mucociliary escalator [14]. Besides this physical barrier function, the epithelium and its components play an important role in the secretion of a wide array of cytokines, chemokines, growth factors and other regulatory mediators when exposed to allergens, pathogens and other stimuli [9, 12]. It has also been proposed that the epithelium can play a role in smooth muscle contraction by the release of soluble mediators released from damaged epithelium [15]. Overall, the epithelium plays an important role in maintaining airway patency throughout the airway tree.

Shedding and desquamation of epithelium has often been described in the medical literature. It is important to note that many of these observations were in endobronchial biopsies and *post mortem* biopsies of large airways in patients who had died from asthma [16–18]. In more recent studies it was suggested that the observed shedding and desquamation could be a result of sampling artefacts, as no differences in biopsies between epithelial shedding or desquamation were observed comparing people with asthma with controls [19]. Moreover, epithelial desquamation increased with decreasing biopsy size [20]. In small airways, epithelial shedding has not been reported [21, 22]. However, increased numbers of columnar epithelial cells in induced sputum from patients with severe asthma have been observed, suggesting increased epithelial cell turnover or shedding in these patients [23]. In general, the epithelium in small airways of people with asthma is more heterogeneous and more varied in thickness [24].

Epithelial cell to cell adhesion, either through tight junctions, adherens junctions or desmosomes, is compromised in the large airways of people with asthma [25, 26]. To our knowledge, no specific studies focussing on the cell-to-cell adhesion in the epithelium of small airways have been conducted. The information provided below is derived from studies that studied epithelial cell-to-cell adhesion in large

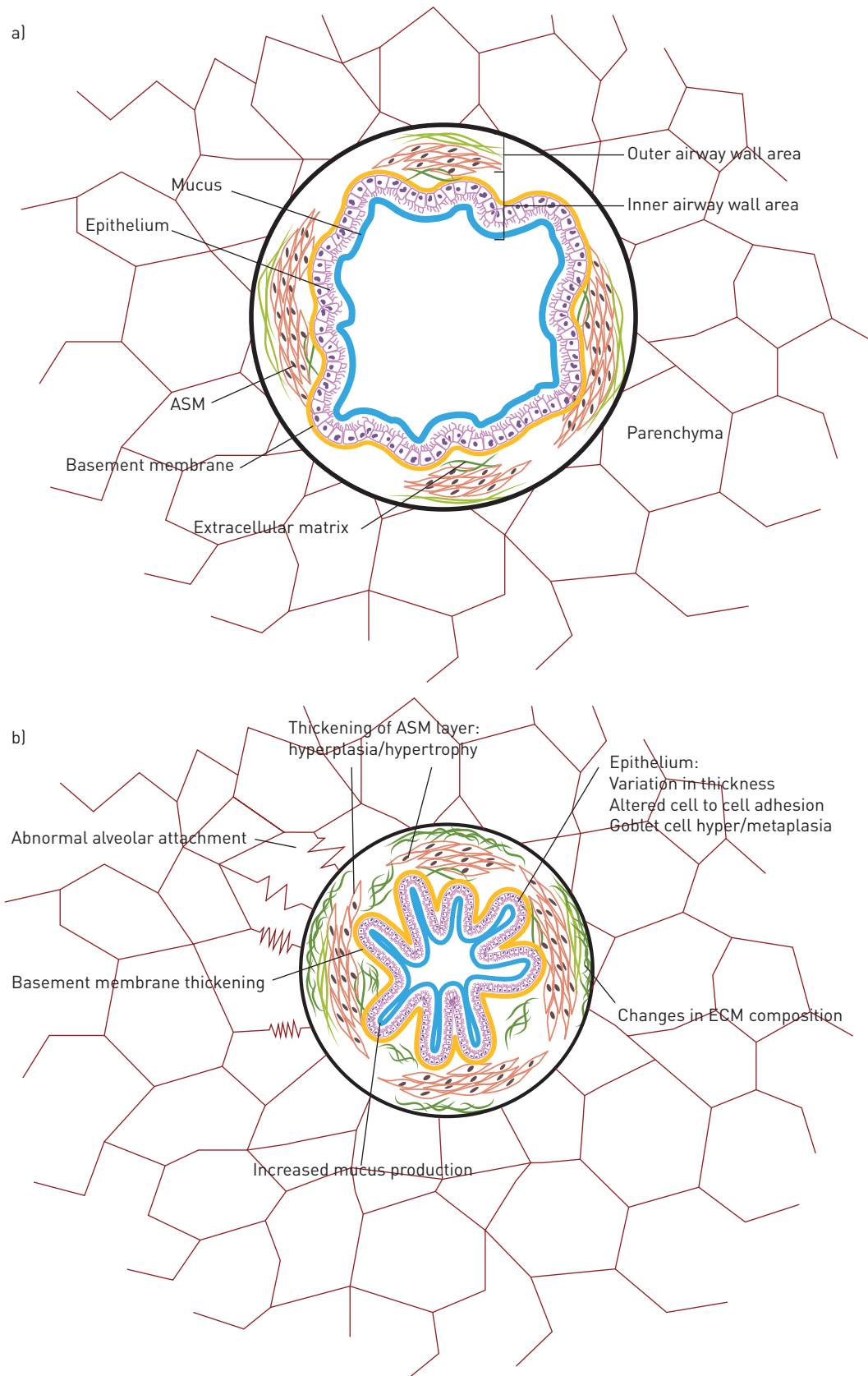


FIGURE 1 a) Schematic drawing of a cross section of a normal small airway embedded in the lung parenchyma. The different compartments and components of the airway wall are shown. b) Schematic drawing of a cross section of a constricted small airway embedded in the parenchyma of a patient with asthma. The structural alterations frequently observed in small airways in asthma are shown. These alterations are located in the epithelium, extracellular matrix (ECM), airway smooth muscle (ASM) and parenchyma. Note that, the ECM is only partly depicted. *In vivo* the ECM forms an extensive network throughout the airway wall.

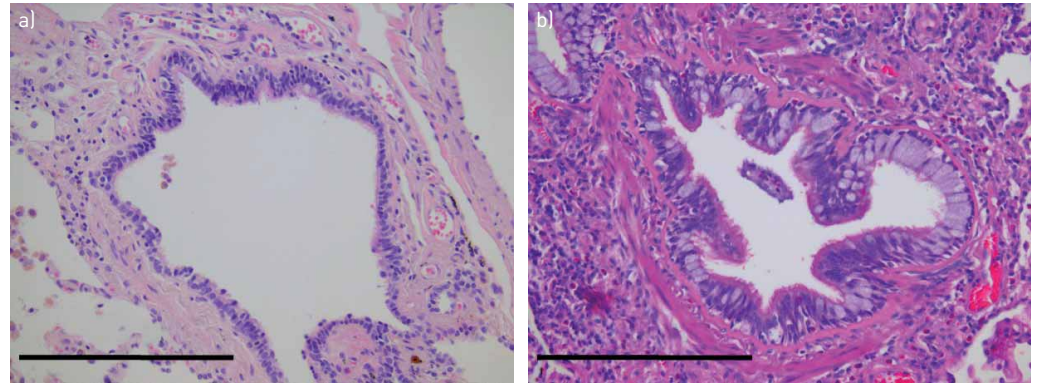


FIGURE 2 Photomicrograph (haematoxylin & eosin stain, magnification $\times 20$) of a) a small airway of a healthy subject and b) a patient with asthma. The small airway of the asthmatic subject shows clear structural alterations to the airway, such as goblet cell metaplasia, reticular basement membrane thickening, airway smooth muscle hyperplasia and eosinophilia. Scale bars=500 μm . Images courtesy of J.H. von der Thüsen (Dept of Pathology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands).

airway biopsy samples. Inflammation is thought to play an important role in the loss of epithelial integrity. Several studies have shown that the expression of proteins involved in cell-to-cell adhesion was decreased when epithelium was exposed to tumour necrosis factor- α [27] or other cytokines [28, 29]. Therefore, exposure to cytokines and other inflammatory mediators might result in structural defects to the epithelium. However, it has been suggested that the fragility of the epithelium of patients with asthma is a fundamental feature of asthma [26] characterised by abnormal repair responses to injury [30].

Normal cell-to-cell adhesion properties of the epithelial cells may also be altered in a process described as epithelial–mesenchymal transition. In this process epithelial cells transition to fibroblast-like mesenchymal cells. This process has been suggested as a means by which abnormal epithelial cells may contribute to the airway wall (including smooth muscle) remodelling observed in asthma [31]. As a result, epithelial cells lose their characteristics and functionality, such as cell-to-cell adhesion [11, 31]. Transforming growth factor (TGF)- β has been shown to play an important role in inducing epithelial to mesenchymal transition. In-depth information about cell-to-cell adhesion dysfunction has been discussed in reviews by LAMBRECHT *et al.* [11] and HACKETT *et al.* [31].

In healthy lungs, goblet cells are only sparsely present in the small airways (figure 2). However, goblet cell hyperplasia (increased number of cells), metaplasia (change in cell phenotype) and mucus accumulation have been frequently described in the small airways of patients with fatal asthma [21, 32–36], severe asthma [37, 38] and in mouse models [39] (table 1). MUC5AC is not seen in healthy small airways but is upregulated in the small airways of patients with asthma [41]. Alterations in expression of MUC5AC may contribute to mucus accumulation and mucus plugging eventually leading to airway obstruction in the small airways of patients with asthma [42]. It has been suggested that tethering of MUC5AC to epithelial mucous cells may further contribute to luminal mucus plugging by impairing mucociliary transport [41]. The increased amount of goblet cells in small airways of people with asthma is most likely a result of goblet cell metaplasia (figure 2) instead of goblet cell hyperplasia [43]. Several possible mechanisms behind goblet cell metaplasia have been suggested. First, trans-differentiation of club and ciliated cells into goblet cells is the main mechanism for goblet cell metaplasia, rather than proliferation of pre-existing goblet cells [11]. Secondly, it is suggested that inflammatory mediators play an important role in the initiation of goblet cell metaplasia [43]. Thirdly, epithelial compression due to bronchoconstriction in asthmatic patients is thought to contribute to goblet cell hyperplasia and metaplasia and subsequent mucus over-production [44].

Many of the functional properties of the epithelium are compromised in patients with asthma. Alterations to the barrier function may lower the protective effect of the epithelium and make the lung more susceptible for inhaled pathogens, pollutants and bronchoconstrictor agents [26]. The impaired mucociliary function and fragile epithelium, in combination with inflammatory cells and increased mucus production, may contribute to luminal narrowing or occlusion by forming mucus plugs, a phenomenon frequently described in small airways of cases of fatal asthma [16, 33, 34, 40]. Although mucus accumulation and plugging has also been observed in small airways of patients with milder and nonfatal asthma [22, 37, 38], we should be cautious to draw general conclusions based on observations in samples of fatal asthma patients. Fatal asthma represents an acute terminal event, associated with inflammatory infiltrates, vascular leakage, severe bronchoconstriction and increased mucus production and accumulation [36, 40].

TABLE 1 Studies of structural epithelial alterations in mild, moderate, severe and fatal asthma

Methods	Population	Findings	[Ref.]
Post mortem specimen: large and small airways (not defined)	Fatal asthma (n=20)	Occluding (mucus) plugs were seen in all small bronchi Epithelial cell shedding was observed in medium-sized bronchi [#]	[16]
Post mortem specimen: segmental and lobar bronchi (Pbm 10>18 mm); subsegmental bronchi (4–10 mm); small and large membranous bronchioles (Pbm <2–4 mm)	Fatal asthma (n=11) Nonfatal asthma (n=13) Control (n=11)	Greater mucus gland area in airways (4–10 mm) in fatal asthma compared to controls No difference in percentage of basement membrane covered with normal, damaged or desquamated epithelium between fatal asthma, nonfatal asthma and controls	[21]
Case report on lung biopsy samples and post mortem specimen: large and small airways (not defined)	Paediatric fatal asthma (n=2) Paediatric asthma in remission (n=2)	In airways >1 mm from patients with asthma in remission mucus plugging, goblet cell hyperplasia and basement membrane thickening was observed; detachment of the epithelial lining was not observed In airways <1 mm from patients with asthma in remission occasional mucus plugs were observed but no goblet cell hyperplasia In the <i>post mortem</i> specimens from patients with fatal asthma similar alterations were observed but more prominent	[22]
Post mortem specimen: large airways (presence of cartilage) and small airways (no presence of cartilage)	Asthma (n=8), of which three were fatal asthma Chronic bronchitis (n=6) Control (n=4)	Significantly more mucus in asthma and chronic bronchitis group than in control group Hypertrophy/hyperplasia of mucous glands and goblet cells in both asthma and chronic bronchitis, with marked hyperplasia of goblet cells in the airways from fatal asthma patients More goblet cells in asthma and chronic bronchitis than in control group but not significant	[32]
Post mortem specimen: large and small airways (not defined)	Fatal asthma (n=93) Control (n=6)	Airways of patients with fatal asthma were more frequently occluded by mucus and cells A greater proportion of cells was present in exudate in the small airways than in larger airways of patients with fatal asthma	[33]
Post mortem specimen: large airways (presence of cartilage) and small airways (no presence of cartilage and <0.4 mm diameter)	Fatal asthma (n=12) Control (n=8)	Increased amount of mucus in fatal asthma in comparison to controls Reticular basement membrane significantly increased in fatal asthma cases compared with controls Median outer luminal diameter was 2mm of large airways and <0.3 mm in small airways	[34]
Post mortem specimen: large airways (presence of cartilage) and small airways (no presence of cartilage)	Fatal asthma (n=3) Nonfatal asthma (n=5) Control (n=4)	Increased amount of mucus in fatal asthma compared to nonfatal asthma and controls Increased amount of mucus in nonfatal asthma compared to controls Increased amount of goblet cells in fatal asthma compared to nonfatal asthma and controls Epithelial thickness in fatal asthma group was higher than in the nonfatal asthma and control group	[36]

Continued

TABLE 1 Continued

Methods	Population	Findings	[Ref.]
Lung biopsy specimen obtained through VATS: large and small airways (not defined)	Severe asthma clinically, with diagnosis of asthmatic granulomatosis (n=10) after histologic evaluation	Papillary epithelial hyperplasia, basal cell hyperplasia and goblet cell metaplasia was observed in all cases Mucus plugging was observed in eight out of 10 cases Subepithelial basement membrane thickening was seen in all cases	[37]
Lung biopsy specimen obtained through VATS: small airways (airways ≤2 mm in size and no presence of cartilage)	Severe asthma (n=29) subdivided into four groups: 1) severe asthma; 2) severe asthma with autoimmune disease; 3) severe asthma with asthmatic granulomatosis; and 4) severe asthma with asthmatic granulomatosis and autoimmune disease	Mucus plugging and goblet cell metaplasia was observed in 20 cases, in cases of severe asthma with asthmatic granulomatosis and autoimmune disease this was observed less frequently than in the other subgroups	[38]
Post mortem specimen: small airways (<2 mm in size and no presence of cartilage)	Fatal asthma (n=6) Control (n=6)	Degree of lumen occlusion observed in all subjects with fatal asthma was significantly higher than controls	[40]

Findings for small airways are presented. Pbm: basement membrane perimeter; VATS: video-assisted thoracoscopy. #: it is unclear whether this is observed in small or large airways.

This severe acute condition is likely to take place on a background of variable levels of inflammation and remodelling affecting different airway wall components, especially airway smooth muscle (ASM) and mucus glands. Findings in fatal asthma samples may, therefore, reflect acute rather than chronic changes. As features of remodelling are also observed in the absence of acute events, they are likely independent. Airway obstruction as a result of mucus plugging and accumulation may lead to impaired gas exchange. It was shown that mucus plugging detected by multidetector computed tomography is associated with large airway obstruction [45]. It is hypothesised that due to their morphology small airways are more vulnerable to mucus plugging. Goblet cell hyperplasia and Muc5ac over-production seen in mice with induced FOXa2 deletion results in increased airway resistance during forced oscillatory ventilation [46]. These results suggest a possible role for the FOXa2 transcription factor in pulmonary diseases with characteristic goblet cell hyperplasia and Muc5ac over-production, whereas in the normal lung FOXa2 is thought to play a role in normal lung development and epithelial cell differentiation. These findings also show the effect of goblet cell hyperplasia and MUC5AC over-production on airway resistance. Goblet cell hyperplasia may result in airway wall thickening and subsequent airway narrowing. In a mouse model, increased epithelial thickness by goblet cell hyperplasia led to more airway closure and airway hyperresponsiveness (AHR) [47].

Extracellular matrix

Across the airway wall, the extracellular matrix (ECM) links cells and contributes to the mechanical properties of the airway. The ECM consists broadly of three components, the basement membrane, the interstitial matrix consisting of a loose fibril-like meshwork and the matrix between cells such as between ASM [48]. The basement membrane is located just below the epithelium (figures 1 and 2) and consists of a basal lamina and the lamina reticularis, often referred to as the reticular basement membrane (figure 1) [49]. The interstitial matrices are intertwined and interconnected with different structures in the airway wall. It is estimated that the ECM consists of around 300 different proteins [50]. However, the five main constituents of the ECM are: collagens, elastic fibres, proteoglycans, matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMPs) [51]. Collagens are the most abundant of the five and have great tensile strength. In contrast, elastic fibres have low tensile strength and high elasticity [51]. Elastic fibres are thought to contribute to elastic properties of the lung. Recently, it was shown that stiffness of the ECM in airways is size dependent, such that in smaller airways the ECM becomes more flexible [52]. Proteoglycans are other important constituents of the ECM and serve multiple functions related to their specific structure. They occupy the spaces between cells and form complexes with other components of the ECM. In addition, they serve as storage for several inflammatory mediators [50]. MMPs and TIMPs are involved in the physiological turnover of the ECM.

The majority of the components of the ECM are produced by fibroblasts and myofibroblasts [53]. However, ASM can also produce ECM constituents [54, 55]. Epithelial cells can stimulate ECM production through

release of periostin which stimulates TGF- β production [11]. TGF- β is the most important protein stimulating the production of ECM components by fibroblasts [53]. The ECM has several functions. First, providing structure and rigidity to the airway wall. Secondly, ECM serves as a reservoir function for a diverse array of inflammatory mediators and growth factors [50]. The ECM, therefore, plays an important role in inflammatory and remodelling processes that occur in asthma [48]. Thirdly, the ECM serves a role in intercellular communication, facilitating cell migration and mechanical force transduction through cell-to-cell or cell-to-matrix adhesion [48, 56, 57]. Although knowledge regarding the ECM has improved during the past years, there are still many questions to be answered, especially concerning the function of ECM.

Compared with controls, both increased and decreased levels of ECM components have been observed in the small airways of patients with asthma and changes in ECM composition are observed within different compartments of the airway wall [58–62] including the inner airway wall area, within the ASM and in the outer airway wall area (table 2). Thickening of the basement membrane is one of the most commonly described structural alteration in the airways of people with asthma (figure 2) [66]. The structural alterations of the ECM are thought to be the result of increased, as well as reduced, production of ECM components by fibroblasts [53, 63] and myofibroblasts [65]. Altered production may be mediated by epithelial cells [67], ASM cells [68] and inflammatory cells [69]. TGF- β is the most important inducer of ECM production and levels of TGF- β have been shown to be increased in asthma patients [70]. More recently, the extracellular protein, fibulin 1c has been suggested as an important regulator of remodelling and inflammation [71].

Alterations in the composition or structure of the ECM in the inner and outer airway wall area [38, 64] may change the biomechanical properties of the airways [51]. Increased collagen content in the ECM may result in stiffening of the airways [52, 72]. Stiffening of the airway wall creates a greater load opposing shortening of the ASM and might have a protective effect against airway narrowing by ASM [73, 74]. Remodelling of the ECM could, therefore, be a mechanism of the airway to counteract increased ASM force. However, remodelling and stiffening might not only oppose smooth muscle shortening. A number of studies have shown that a deep inspiration in patients with asthma does not have the same bronchodilation or bronchoprotective effect as it does in people without asthma [75, 76]. Although the mechanism behind the bronchoprotective and bronchodilating effects of a deep inspiration in people without asthma is not clear, it is thought to depend on the refractoriness of the ASM, relative to the surrounding lung parenchyma after it is lengthened. This lengthening or stretching upon inhalation subsequently relates to the compliance of the airway. In other words, a change in transpulmonary pressure, due to lung inflation and deflation, causes ASM to lengthen or to shorten. Increased stiffness of the airway wall could result in less transduction of force to the ASM, impaired bronchodilation during a deep inspiration or tidal breathing and increased susceptibility to bronchoconstriction. A study using bronchial biopsy samples showed an association between deep inspiration-induced bronchodilation and certain components of the ECM [77]. Moreover, a recent study showed that increased stiffness of the ECM by itself has been shown to increase force generation by ASM cells and change ASM cell interconnectivity [52]. In fatal asthma, an association between decreased elastic fibre content and the number of abnormal alveolar attachments has been observed [59]. The parenchyma exerts outward tethering forces on the airway wall of the airways through airway–alveolar attachments. These outward tethering forces contribute to the patency of the airway. Airway–alveolar uncoupling as a result of structural changes in the outer airway wall will result in diminished tethering forces and contribute to increased airway narrowing by ASM shortening [59, 71, 78]. Uncoupling could also result in less force transduction to the ASM, resulting in less strain and subsequent relaxation of the ASM.

Increased inner airway wall area due to thickening of the reticular basement membrane and the submucosa will contribute to excessive airway narrowing as the surrounding ASM shortens due to encroachment of the airway wall on the airway lumen [79]. In a study using endobronchial biopsies, relationships between structural alterations of the ECM within the ASM, especially collagen I, and clinical indicators of bronchodilation and bronchoconstriction have been observed [80]. However, to our knowledge no studies have been reported linking structural alterations of the ECM in small airways of people with asthma to spirometry indices of peripheral obstruction.

ASM

Asthma is characterised by excessive and reversible airway narrowing (or AHR). AHR is demonstrable in the lung function laboratory by administering inhaled drugs, allergens or stimulants that result in ASM contraction and can be reversed in the laboratory or clinically by drugs that relax ASM. Not surprisingly therefore, the ASM is probably the most discussed and investigated constituent of the large and small airways in asthma. Despite extensive research there are still a lot of unanswered questions regarding the structure and function of ASM in small airways of patients with asthma. In the trachea, the transverse ASM layer is limited to the membranous posterior wall. After bifurcation of the trachea the smooth

TABLE 2 Studies of extracellular matrix composition in mild, moderate and severe asthma

Methods	Population	Findings	[Ref.]
Post mortem specimen: large airways (Pi >6 mm) and small airways (Pi <6 mm)	Fatal asthma (n=18) Control (n=10)	Inner airway wall area: proteoglycans (versican increased) Outer airway wall area: proteoglycans (decorin and lumican decreased)	[58]
Post mortem specimen: small airways (Pi <6 mm)	Fatal asthma (n=15) Control (n=9)	Outer airway wall area: elastic fibre content decreased	[59]
Post mortem specimen: two large airways (Pi >6 mm) and three small airways (Pi <6 mm) from each subject	Fatal asthma (n= 35) Nonfatal asthma (n=10) Nonasthmatic control (n=22)	Within ASM: fractional area of elastic fibre was increased in fatal asthma <i>versus</i> nonfatal asthma, but not to nonasthmatic control Collagen I and III, fibronectin, versican, MMP (1, 2, 9 and 12) and TIMP (1 and 2) were not significantly different in fatal asthma <i>versus</i> nonasthmatic control	[60]
Post mortem specimen: central versus peripheral airways were defined according to presence of cartilage	Fatal asthma (n=31) Control (n=10)	Inner airway wall area: elastic fibre content nonsignificant	[61]
Post mortem specimen: large airways (Pi >6 mm) and small airways (Pi <6 mm)	Fatal asthma (n=24) Control (n=11)	Inner airway wall area: collagen type I increased, collagen type III decreased Outer airway wall area: collagen type III decreased and collagen type I, fibronectin, MMP-1, MMP-2, MMP-9 all increased TIMP 1 and 2 were not significantly different Collagen expression increased	[62]
Transbronchial biopsy specimen: alveolar tissue	Mild asthma (n=11) Control (n=12)	Proliferation rate of distally derived fibroblasts decreased Versican increased, biglycan and decorin decreased, and perlecan was nonsignificant from distally derived fibroblasts	[63]
Surgical lung biopsies: small airways and alveolar parenchyma	Severe asthma only (n=5) Severe asthma with autoimmune disease (n=5) Severe asthma with asthmatic granulomatosis (n=9) Severe asthma with asthmatic granulomatosis and autoimmune disease (n=10)	Submucosal fibrosis and subbasement membrane thickening was observed	[38]
Post mortem specimen: small airways (Pi 2–10 mm)	Fatal asthma (n=25) Control (n=11)	Inner airway wall area: increased collagen	[64]
Bronchial and transbronchial biopsies: alveolar parenchyma	Uncontrolled atopic asthma (n=16) Controlled atopic asthma (n=9) Control (n=8)	Increased collagen in uncontrolled atopic asthma <i>versus</i> control Decreased versican in controlled atopic asthma <i>versus</i> control Increased decorin in uncontrolled atopic asthma <i>versus</i> control Decreased biglycan in controlled atopic asthma <i>versus</i> control and uncontrolled atopic asthma Decreased MMP-9 in controlled atopic asthma <i>versus</i> control and uncontrolled atopic asthma Increased TIMP-3 in uncontrolled and controlled atopic asthma <i>versus</i> control Decreased myofibroblasts in controlled atopic asthma <i>versus</i> control and uncontrolled atopic asthma Fibronectin: nonsignificant difference between the three groups	[65]

Findings for small airways are presented. Pi: perimeter; ASM: airway smooth muscle; MMP: matrix metalloproteinase; TIMP: tissue inhibitors of matrix metalloproteinase.

muscle encompasses an increasingly greater proportion of the airway circumference in an essentially transverse orientation with only a few degrees of spiral orientation, down to the membranous bronchioles (figure 1) [81]. Although the absolute volume and thickness of the ASM layer is decreased in small

airways, its thickness in relation to total airway wall volume increases [82]. The amount of ASM in the airway wall diminishes in the terminal bronchioles although contractile elements persist even in the lung parenchyma, mostly at the alveolar openings. It should be noted that the smooth muscle layer consists not only of smooth muscle but also of ECM, blood vessels, resident mast cells and (few) inflammatory cells [83].

In people with asthma the structure of the ASM layer is altered. This is most clear in the large airways but may also be seen in the small airways (figures 1 and 2) [7, 21, 35, 44, 60, 71, 79, 84, 85]. Although many studies have observed increased thickness of the ASM layer in the small airways, based on the sampling to date, it appears that this occurs in <50% of cases. In a study in fatal paediatric asthma a nonsignificant increase in thickness of the ASM in the large airways was observed but no increase in ASM thickness of the small airways [34]. This was also observed in adults with fatal asthma where there was no significant difference in ASM area, compared with control cases, in very small airways (<2 mm internal perimeter) [86]. In a study of 68 controls and nonfatal and fatal cases of asthma, ELLIOT *et al.* [7] found that 41% of asthma cases had small airways with increased thickness of the ASM layer. In only 6% of the cases was the increased ASM thickness observed in the small but not the large airways. These observations point to heterogeneity of ASM thickening throughout the bronchial tree in asthma. Capturing this heterogeneity is much “easier” in the large airways given their relatively smaller number and accessibility. Sampling even twice as many small airways is still a tiny sample of the total number of small airways in the lung periphery [3] and therefore less likely to be representative of the heterogeneity in the small airways. Studies examining the effect of increased sampling of small airways will provide some insight into the degree of heterogeneity.

The increased thickness of the ASM layer is thought to be the consequence of a mix of hypertrophy, hyperplasia (figure 2), increase in inflammatory cells, angiogenesis and increased deposition of the ECM [83]. A number of studies have shown hypertrophy and hyperplasia of ASM cells of large and small airways in patients with asthma (figure 2) [7, 16, 38, 85, 87, 88] or in an equine model of asthma [89]. Localisation of either hypertrophy and/or hyperplasia differed between these different studies. Several studies reported ASM hypertrophy in the small airways of cases of fatal asthma [16, 85]. In contrast another study showed hyperplasia of the small airways of patients with fatal asthma [87]. The heterogeneity in these findings could be the result of variation in techniques to assess the structure of the ASM layer (*e.g.* variation in airway section thicknesses) [83]. Although the overall consensus is that the thickness of the ASM layer is increased in patients with asthma, the origin of this pathology is still uncertain. One hypothesis is that the increased smooth muscle is a result of the growth promoting effects of chronic airway inflammation. A number of studies have therefore investigated the degree of proliferation ASM cells *in vitro* and *in situ* [90–94]. The results of these studies have been contradictory not only in the degree of proliferation observed in control cases and the severity of asthma cases but also in the differences between case groups with and without asthma. However, the largest study found no difference in proliferation between controls and mild and severe cases of asthma or any constant relation of proliferation with cell number or airway inflammation, in either large or small airways [92]. Other mechanisms such as decreased apoptosis of ASM cells, migration and trans-differentiation of non-ASM cells (*e.g.* epithelial–mesenchymal transition) have also been suggested to be possible mechanisms leading to ASM hyperplasia [31, 93, 95]. Alternatively, it is possible that increased ASM is independent of inflammation [96] and may be present from early life as suggested by the absence of a relationship between increased ASM and duration of asthma [97], the presence of increased ASM in preschool children who later develop asthma [98] and the studies that show abnormal airway function within the first months of life in infants who later develop asthma, for example [99].

Over the years there has been quite extensive debate on the functional role of ASM in healthy lungs [100–105]. Some authors are not convinced that ASM has an important physiological function and have referred to the ASM as being like the “appendix of the lung” or being “a frustrated cell” [104, 105]. These no-function believers state that there are no pulmonary diseases related to the loss of contractile function of ASM and therefore it has no function [104]. In addition, it is suggested that, as asthma patients who undergo bronchial thermoplasty do not have lasting clinical problems, the ASM does not have a physiological function [102]. Other authors have proposed several possible functional properties of ASM contractility within the airways. For example, it was suggested by MEAD [100] that contraction or relaxation of ASM might be involved in mucus clearance by adjusting the thickness of airway surface liquid layer. Moreover, it has been suggested that *in utero* peristaltic contraction of the ASM may contribute to lung growth [106]. These are only two of the many roles proposed for the contractile function of the ASM [100, 102, 103]. In a review published by CIERI [107], it has been suggested that ASM in the terminal bronchiole of mammals plays an important role in keeping the alveoli inflated at low lung volumes and that ASM might attenuate ventilation–perfusion heterogeneity by constricting airways in either the apex or base in different situations. Furthermore, the ASM is assigned an important role in the production of cytokines, chemokines, growth factors and ECM components [108]. Therefore, considerable debate remains not only

about the normal physiology of ASM but also about the origins of the increased ASM seen in patients with asthma.

The characteristic symptom cluster in asthma is variable cough, shortness of breath, chest tightness and/or wheeze. Physiologically this manifests as AHR (excessive and reversible airway narrowing) in response to contractile stimuli that activates ASM. Over the years, many studies have been conducted to examine the role of ASM in causing excessive airway narrowing. The majority of these studies were conducted *in vitro* using samples of large human airways or with animal airways. It has been shown that ASM of humans is in certain ways comparable to that of animals [109] and therefore the result of these studies can be used to draw conclusions regarding ASM in humans. There are only a few studies focussing on the mechanical properties of ASM in small airways of patients with asthma, mainly due to the difficulty of obtaining lung tissue samples from patients with asthma and the technical challenges of studying the ASM of the small airways in isolation *in vivo* due to their size and difficulty of access. However, we identified some studies that focussed on the ASM of the small airways in asthma which we will discuss below.

Increased contractility

An early hypothesis of the mechanism of the AHR observed in patients with asthma, was that the ASM intrinsically generates higher contractile force and therefore could narrow the airways more easily. In the studies of the larger airways of people with asthma there is conflicting evidence regarding the difference in maximal contractile force between patients with and without asthma [109–114]. The majority of these studies showed no difference between groups with and without asthma. In small airways the majority of studies showed no difference in contractile force either [115–117]. However, in one study the contractile response of ASM from a single patient with asthma was significantly higher compared with ASM from people without asthma [118].

Adaptation

A process that might be related to ASM-induced airway narrowing is called length adaptation or ASM plasticity. This is the ability of ASM to generate and maintain optimal force by adapting its contractile function to different lengths [119]. In people without asthma, length adaptation of the ASM is continuously perturbed by the dynamic movement of the lung during normal breathing. In patients with asthma it is thought that there are fewer length fluctuations that perturb the force-generating capacity due to stiffening or (partial) airway–alveolar uncoupling, as mentioned earlier in the ECM section [119].

Force adaptation is another mechanism through which the contractile force can be increased. This characteristic of ASM was first described by BOSSE *et al.* [120]. The authors showed that in segments of sheep trachea increased ASM basal tone, induced by exposure to acetylcholine, led to a time-dependent increase in total contraction force of ASM [120]. In a subsequent modelling study, they showed that force adaptation may lead to increased airway narrowing in any generation [121], and that this force adaptation is reversible after removing the contractile agonist. It is suggested that inflammation-derived spasmogens in people with asthma result in increased basal tone of ASM. This increased basal tone could subsequently lead to force adaptation and eventually excessive bronchoconstriction.

Increased maximal shortening or velocity of shortening

Besides alterations in contraction force, other properties of the ASM in asthma might play a role in excessive airway narrowing. It was suggested that increased maximal shortening of ASM might be such a feature. MITCHELL *et al.* [122] observed that in passively sensitised bronchial rings of peripheral airways, maximal ASM shortening was significantly greater in patients with asthma compared with individuals without asthma. In ASM obtained through endobronchial biopsies of patients with asthma [123] and an asthma model using sheep trachea [124] it was observed that there was increased maximal shortening of the ASM. However, in another study in large airways in patients with asthma no difference in maximal shortening was observed [109].

The study of MITCHELL *et al.* [122] also found that the shortening velocity of ASM was significantly increased in peripheral airways. In an equine model of asthma [125] it was shown that maximal shortening velocity of peripheral ASM was significantly greater compared with controls and compared to that of their tracheal ASM. The majority of studies of large airways have shown no differences in shortening velocity between people with and without asthma [110, 115]. However, in another study it was shown that shortening velocity of bronchial smooth muscle cells from the large airways of patients with asthma was significantly increased [123]. SOLWAY *et al.* [126] postulated that possible alterations in shortening velocity might affect the ASM relaxation by deep inspiration. They reasoned that increased shortening velocity of the ASM in patients with asthma allows the ASM to return to its pre-stretched state more quickly and thus shorten more in response to a contractile stimulus [126].

As discussed in the ECM section the bronchodilation or bronchoprotective effect of a deep inspiration is impaired in patients with asthma [75, 76]. A study of bovine lungs showed that small airways are two times more compliant than larger airways when subjected to transmural pressure oscillation [127]. In this study, increased compliance of the small airways led to a greater strain of the ASM and subsequently a greater recovery after induced bronchoconstriction [127]. The impaired ability to impose a strain on the ASM in patients with asthma could be the result of stiffening or (partial) airway–alveolar uncoupling. The strain imposed on the ASM in order to stretch and relax the ASM is not sufficient to fully reverse induced bronchoconstriction [128]. Therefore, these results suggest that in addition to abnormalities to the ASM other factors might be of importance in the mechanism that leads to excessive bronchoconstriction observed in patients with asthma.

ASM mass

The increase in ASM mass in patients with asthma [16, 21, 38, 40, 84, 85, 87, 97, 129] and its related force generation is potentially the most important contributing factor to the excessive airway narrowing observed in asthma. The increased maximal airway narrowing in patients with asthma is induced by stimuli that contract ASM directly or indirectly and are reversed by stimuli that relax ASM [130]. It has been shown in a computer model that ASM contraction contributed the most to increased resistance after induced bronchoconstriction [131]. *Ex vivo* studies of airway segments from patients with and without asthma have shown that the volume of ASM within the airway wall was related to maximal airway narrowing [132] demonstrating that the remodelled ASM retains its contractile properties and is capable of overcoming potentially increased loads opposing shortening in a remodelled airway. In addition, narrowing of the airway lumen during even normal shortening of the ASM will be exaggerated by the space-occupying effects of the remodelled airway wall (including the ASM) [86]. This effect was estimated to result in a 37-fold increase, compared with controls, in the resistance of airways <2 mm, despite no significant difference in ASM mass [86]. Another study showed that a decrease in ASM mass caused the ASM to behave the same as a deep inspiration as in people without asthma [124]. There are multiple individual studies showing that both the airway response to an inhaled smooth muscle agonist (histamine or methacholine) and the level of sensitivity to a specific allergen, independently predict the airway response to the sensitised inhaled allergen [133, 134], analogous to experimental findings in an animal model [96]. Therefore, although the evidence to suggest that the ASM is “bad” [121] is contentious, there is ample evidence that the increased ASM in asthma is “bad news” and that it likely requires a “bad environment” for the increased ASM to manifest as excessive airway narrowing. This environment is most likely inflammation driven by allergy, as allergy is the most powerful predictor of asthma onset in childhood [135].

Lung function tests to assess small airways disease

There are several lung function tests that can be used to assess small airway function in pulmonary disease. Spirometry is the most commonly used technique to measure lung function in daily clinical practice and is well standardised, widely available and highly reproducible. Forced expiratory volume at 1 s, which is often used as an end-point in clinical trials, is not very sensitive to small airways changes. Spirometric indices that are purported to be more sensitive to changes in the small airways are forced expiratory flows at 25–75% and 75% of forced vital capacity. A different way to assess small airway function in patients with asthma is by using washout tests. The single- or multiple-breath washout test assesses ventilation distribution inhomogeneity by examining the clearance of an inert gas, most often nitrogen, over single or multiple breaths [128]. The indices that are derived from the washout curve (e.g. lung clearance index and index of conductive or index of acinar ventilation inhomogeneity) may help localise and identify the structural changes that contribute to ventilation inhomogeneity. Washout tests are reproducible and sensitive to minor changes. A relatively new technique to assess resistance in the small airways is by the use of impulse oscillometry. With this technique oscillations of airflow at different frequencies are used to determine the resistance in the central and peripheral lung, including the small airways. These oscillations are generated by a loudspeaker and the resulting pressure and airflow changes are measured at the mouthpiece. Impulse oscillometry is an easy to use, noninvasive and effort-independent technique. The diagnostic accuracy of these techniques to assess the small airways independent of large airway disease is still debated and there is still no generally accepted gold standard. For more in-depth information regarding the aforementioned techniques to assess SAD and the diagnostic accuracy of these measurements we refer to several extensive reviews on this topic [136, 137].

Imaging and modelling

Most of the aforementioned research conducted on the small airways studied the behaviour of isolated components of the small airways *in vitro*. Over recent years, imaging methods have been increasingly used and developed to gain knowledge on structure and function of the small airways in the lung *in vivo*. By combining the findings of isolated components and imaging modalities into models, researchers have tried

to derive and predict the behaviour of the small airways *in vivo*. This approach has led to new and important insights regarding the small airways.

Static imaging

In its early days, chest computed tomography (CT) was not able to visualise small airways because their size was below the resolution of the CT scanners. Thanks to the development of high-resolution CT scanners and advanced reconstruction techniques it is now possible to visualise airways in the range of 1 mm in diameter using low-dose protocols. CT images acquired during a breath hold at full inspiration can provide detailed information on airway generation and airway wall dimensions (figure 3) [138]. In operated patients with COPD it was shown that airway size measured by pre-operative CT correlated to airway size as measured histologically [139]. In asthma it was shown that the airway walls, as measured on CT, were thicker compared to healthy subjects. Furthermore, it was shown that increased airway wall thickness observed on CT was related to lower forced expiratory volume in 1 s and higher risk of exacerbations [138, 140]. Static CT images acquired during expiration can show regions of low attenuation that are thought to reflect SAD (figure 2). These low-attenuation regions depict areas of gas trapping and/or hypoperfusion, due to premature closure of small airways during expiration and reduced ventilation leading to hypoperfusion. In clinical studies in patients with asthma, low-attenuation regions have been associated with disease duration, asthma exacerbations, airflow obstruction and inflammation [141]. Hence, low-attenuation regions can be used in clinical studies as an outcome measure to evaluate efficacy of treatment targeting the small airways [142]. Well-standardised volume-controlled CT protocols and the development of sensitive automated image-analysis systems to measure airway dimensions and low-attenuation regions are needed to further facilitate the use of chest CT-related outcome measures in clinical studies. A limitation of chest CTs is that they expose the patient to ionising radiation, which restricts the number of CTs that can be made for monitoring airway dimensions and low-attenuation regions in response to therapies and furthermore for studying the lungs dynamically.

Dynamic imaging

Magnetic resonance imaging (MRI) is a radiation-free alternative to CT that is able to provide us with information on lung structure and function. Ventilation defects or ventilation heterogeneity can be visualised with MRI using hyperpolarised gases [143] or after expiration to residual volume level. Other imaging modalities, such as single-photon emission CT and positron emission tomography, can also be used in ventilation imaging. Ventilation defects are thought to represent regions of heterogeneous and abnormal gas or air distribution. It is hypothesised that ventilation heterogeneity or defects correspond to regions in the lung that are not well-ventilated [144]. It has been shown that the presence of ventilation defects, frequently observed in patients with asthma, is due to heterogeneous airway closure or narrowing of small airways [145]. In several studies it was shown that ventilation defects are not seen randomly but they might be predefined [146] or a result of remodelling [147]. Furthermore, it has been shown that ventilation defects persist over time [146, 148, 149]. Ventilation defects in patients with asthma could resolve after induced bronchoconstriction most likely as a result of redistribution of airflow, resulting in poorly ventilated regions receiving more airflow and *vice versa* [148]. It was suggested that if ventilation defects persist over long periods, one would expect that atelectasis would occur in these regions [149].

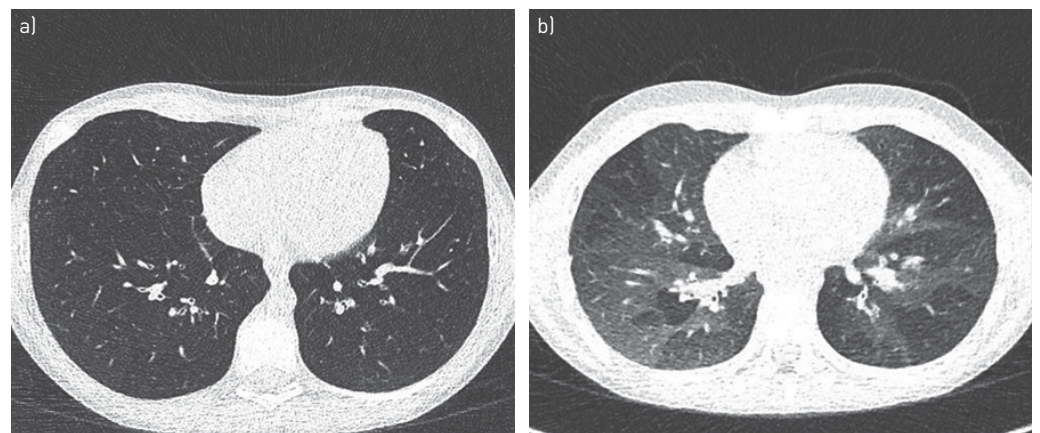


FIGURE 3 Spirometer-guided chest computed tomography (CT) in a patient with severe asthma. a) Inspiratory chest CT showing clear airway wall thickening. b) Expiratory chest CT in the same patient showing extensive regions of low attenuation.

TABLE 3 Summary of possible structural and functional changes in the small airways of asthmatics

	Structural changes	Functional changes
Epithelium	Variation in thickness Altered epithelial cell to cell adhesion Goblet cell hyper/metaplasia Mucus accumulation/plugging Alterations in expression of MUC5AC	Impaired barrier function Increased mucus production Epithelial–mesenchymal transition
ECM		
Inner airway wall area	Changes in ECM composition Basement membrane thickening	Altered ECM production Stiffening of the airways Increased ASM opposing force
Within ASM	Changes in ECM composition	Altered ECM production Stiffening of the airways
Outer airway wall area	Changes in ECM composition Outer airway wall area thickening	Altered ECM production Stiffening of the airways Increased ASM opposing force Decreased force transduction
ASM	Thickening of ASM layer ASM hyperplasia/hypertrophy	Increased contractile force Length adaptation Force adaptation Increased maximal shortening Increased shortening velocity Impaired bronchodilating and protective effect after a deep inspiration
Parenchyma	Abnormal airway alveolar attachments	Decreased force transduction Decreased parenchymal tethering force

ECM: extracellular matrix; ASM: airway smooth muscle.

Therefore, some sort of partial or intermittent closure must be present in the patients with persisting ventilation defects or these areas are ventilated through collateral ventilation [150]. Ventilation defects are associated with disease severity [144, 147, 151, 152], lung clearance index [152] and spirometry [144, 151].

An in-depth review by KING *et al.* [140], reported important findings on CT and three-dimensional ventilation imaging in the large airways, small airways and the parenchyma of patients with asthma. In that review, KING *et al.* [140] further elaborate on the current and possible future use of different imaging modalities to improve our understanding of the role of small airways in asthma pathophysiology and on what further research is needed.

Modelling

Over a number of years efforts have been made to explain the emergent behaviour of airways leading to ventilation defects in asthma. Based on a single airway model by ANAFI *et al.* [153], VENEGAS *et al.* [145] extended this model and discovered that behaviour of an individual airway affects the whole airway tree and *vice versa*. A further extension of this model by WINKLER *et al.* [154] proposed a mechanism for the emergence of ventilation defects. The model describes a vicious circle based on interdependence of airways. In short, they show that if bronchoconstriction reaches a critical point in which airways become unstable, then any change in airflow causing a reduction in airflow in the daughter airway leads to a smaller tidal volume. Subsequently, reduced expansion of the adjacent lung parenchyma increases airway constriction and airway resistance, which in turn leads to a greater reduction in airflow through the airway relative to parallel airways. This leads to a vicious circle in which bronchoconstriction further increases. This model is a step forward in understanding the complex behaviour of airways mechanics in asthma exacerbations. Therefore, it is possible that localised areas of remodelling that result in excessive airway narrowing will alter behaviour of airways in other parts of the airway tree. These complexities, including the heterogeneity of airway remodelling need to be considered when observing behaviour of the lung in asthma. Future modelling studies, combining morphologically, clinically and imaging-derived parameters on structure and function with computer simulation will improve our understanding of airway mechanical behaviour.

Conclusion

The European Respiratory Society International Congress in 2019 in Madrid, Spain, once again highlighted the importance of the need to increase knowledge on the small airways in asthma. The high

prevalence of small airways involvement in people with asthma observed in the ATLANTIS study accentuates this need [6]. This review article provides us with an overview of the structural and functional alterations observed in the small airways of patients with asthma (table 3). Asthma is a heterogeneous condition and therefore, it is unlikely that there is one overarching mechanism that will do justice to the complexity of its pathophysiology. Since Hippocrates first used the term asthma our ideas and understanding on pathophysiology, diagnostics and treatment of asthma have changed, revealing a number of possible phenotypes covered by the term asthma. SAD in asthma could be such a phenotype which deserves further study.

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