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SERIES "AIRWAY REMODELLING: FROM BASIC SCIENCE TO CLINICAL PRACTICE"
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Tools used to measure airway remodelling in research

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ABSTRACT: Airway remodelling refers to changes in the airway structure and includes subepithelial fibrosis, increased smooth muscle mass, submucosal gland enlargement, neovascularisation and epithelial alterations. Remodelling is observed in response to chronic injury and is seen not only in asthma but in all airway diseases.

Remodelling is associated with more severe airflow obstruction and airway hyperresponsiveness in asthma; however, the clinical significance of this is still a matter of debate. Research should be pursued to better understand the accurate implication of airway remodelling in disease and its therapeutic modulation.

To allow research in this field, accurate and standardised methods should be utilised to measure airway alterations in disease and following therapy. The standard detection of structural alterations is through direct analyses of airway tissues obtained during a *post mortem*, surgically or by flexible bronchoscopy. To avoid invasive techniques, other tools have been developed to indirectly measure remodelling, including induced sputum, bronchoalveolar lavage fluid, blood and urine analyses, physiological and radiological assessments, as well as *in vitro* techniques.

Although of great interest, the exact significance of airway remodelling measurements gained through such indirect techniques is uncertain and further research is needed. Despite their invasive nature, direct methods should be favoured to adequately measure airway remodelling in disease and its modulation by therapy.

KEYWORDS: Airway remodelling, asthma, chronic obstructive pulmonary disease, immunohistochemistry, pathology

Airway remodelling includes subepithelial fibrosis, increased smooth muscle mass, enlargement of glands, neovascularisation and epithelial alterations. Remodelling is not only restricted to the airways but also occurs in a wide range of tissues and organs, including the skin in sclerodermia [1] and wound healing process [1, 2], as well as in the intestine in inflammatory bowel diseases [3, 4]. Remodelling is observed in almost every injured tissue, particularly in tissues that undergo repeated chronic injury. In airway diseases, remodelling is associated with clinical outcomes such as more severe airflow obstruction and airway hyperresponsiveness [5–9]. The clinical significance of airway remodelling is still controversial. However, targeting airway remodelling with treatment has the potential to decrease severity, to improve control and to prevent disease expression. To better understand the accurate implication of airway remodelling in diseases and its modulation by therapy, more research should be performed. The present manuscript is an overview of the current techniques used to assess airway remodelling.

REMODELLING IN AIRWAY DISEASES

Airway remodelling in asthma

Airway remodelling in asthma was first described in cases of fatal asthma in 1922 by H.L. Huber and K.K. Koessler (reviewed in [10]). Airway remodelling has been documented in

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not only the large but also in small asthmatic airways [11]. Loss of epithelial integrity [12], thickening of basement membrane [13], subepithelial fibrosis [14], goblet cell and submucosal gland enlargement [15, 16], increased smooth muscle mass [15], decreased cartilage integrity [17] and increased airway vascularity are the structural changes that have been documented in the asthmatic airways [18, 19]. Epithelial alterations in asthma are epithelial fragility and goblet cell hyperplasia. It is still a matter of debate whether epithelial detachment is a real disease phenomenon or simply an artefact of biopsy sampling [12, 15, 20, 21]. Subepithelial fibrosis is seen at the level of lamina reticularis and this fibrotic process may extend deeper into the subepithelial layer. Fibrosis is a result of increased deposition of extracellular matrix (ECM) proteins, including collagens I, III and V, fibronectin, tenascin, lumican and biglycan [13, 22–25]. Alterations in smooth muscle layer are hypertrophy, hyperplasia and migration of the smooth muscle cells to the subepithelial area [15, 26, 27]. The airway smooth muscle cells may participate in enhancing inflammation and remodelling through the release of cytokines, chemokines and ECM proteins [28–30]. Vascular alterations include increased size of airway wall vessels and angiogenesis [18]. These structural changes may stem from an ongoing chronic inflammatory process involving the activation of inflammatory cells, including CD4+ T-cells, eosinophils, neutrophils and mast cells [31–35]. The inflammatory mediators that drive this process include cytokines (interleukin (IL)-4, IL-5, IL-9 and IL-13), transforming growth factor (TGF)-β, granulocyte/macrophage-colony stimulating factor, lipid mediators and histamine. Some of these mediators have potent remodelling properties, such as TGF-β, IL-11 and IL-17 [5, 36, 37].

Structural alterations in chronic obstructive pulmonary disease

Airways of patients with chronic obstructive pulmonary disease (COPD) are characterised by squamous cell metaplasia, loss of epithelial cilia, goblet cell hyperplasia, mucus gland enlargement and smooth muscle hypertrophy [38–41], with increases in smooth muscle mass more predominant in smaller than larger airways [42]. Airway wall fibrosis and stenotic lesions are also observed in the small airways [43] and imbalance between profibrotic proteases and protective antiproteases has been implicated in the development of emphysema [44]. Normally, basement membrane thickening is not characteristic of airway remodelling in COPD patients, although it has been reported in a subgroup of COPD patients who have a predominant eosinophilic inflammatory profile [45]. Angiogenesis is also observed in COPD airways [46]. In COPD, inflammation is characterised by increased macrophages, CD8+ T-cells and neutrophils and is largely driven by IL-8, tumour necrosis factor-α, leukotriene (LT)B_4 and TGF-β [39, 47–53]. Many, if not all, of these structural changes in the COPD lungs can be attributed to direct injury and inflammation from cigarette-smoke components.

Structural alterations in other airway diseases

Bronchiectasis

Bronchiectasis refers to an abnormal dilatation of the bronchi attributed to airway wall destruction strongly related to post-infectious processes. In bronchiectasis, destruction of bronchial cartilage and smooth muscle layer, bronchial ulceration and obstruction of airways by granulomas are common [54]. Otherwise, little is known on detailed airway abnormalities.

Cystic fibrosis

Cystic fibrosis (CF) is a genetic disorder involving mutations of the CF transmembrane regulator gene. In the CF lung, bronchiectasis, bronchioloectasis and fibrotic changes are often seen. The structural alterations characterising the CF airways include goblet cell and submucosal gland extension to bronchioles and greater height of the epithelium. Loss of cartilage [55], excessive angiogenesis [56] and increased thickness of inner wall and smooth muscle areas in peripheral airways is also observed [57]. A dense fibrous deposition of collagens I and III, tenascin and elastin is reported in the bronchial wall [58]. CF airways are infiltrated with neutrophils, as well as B- and T-cells [59]. IL-8, a neutrophil chemoattractant, and LTB_4 are the main inflammatory mediators in CF [60].

As summarised in table 1, most of these structural changes are not characteristic of one specific airway disease, but are shared by all airway diseases. These similarities suggest that the excessive repair process in the human airway is a common way in which to resolve chronic miscellaneous injuries.

METHODS FOR ASSESSMENT OF AIRWAY REMODELLING

Table 2 presents the tools used in the methods of assessing airway remodelling discussed in the following sections. Direct assessment of remodelling and Indirect assessment of remodelling.

Direct assessment of remodelling: airway tissues

Post mortem and surgical lung specimens

Pathological features of airway disease were first described in lung specimens obtained by surgery or autopsy. Surgical

TABLE 1 Features of airway remodelling in airway disease

	Epithelial alteration	RBM thickness	Subepithelial fibrosis	Mucus gland hyperplasia	Smooth muscle mass	Angiogenesis
Asthma	Detachment	+++	+++	++	+++	+++
COPD	Metaplasia	+	++	+++	++	+
CF	Densely ciliated	++	++	+++	++	++

Each + represents the degree of association with disease. RBM: reticular basement membrane; COPD: chronic obstructive pulmonary disease; CF: cystic fibrosis.

TABLE 2 Tools used to assess airway remodelling

	Epithelial alteration	ECM deposition	Mucus glands	Smooth muscle mass	Angiogenesis
Airway tissue					
Lung specimen, EBB, TBB	H&E	Van Gieson, Sirius Red M-T, IHC	H&E, PAS	H&E IHC for smooth muscle α-actin	IHC for collagen IV or Von Willebrand factor
Airway fluid					
BALF, IS	Indirect *via* number of BEC	ELISA or RIA for collagen products, MMP/TIMP	NA	NA	ELISA for VEGF and endostatin
Blood/urine	NA	Collagen products, MMP/TIMP	NA	NA	?

ECM: extracellular matrix; EBB: endobronchial biopsy; TBB: transbronchial biopsy; BALF: bronchoalveolar lavage fluid; IS: induced sputum; H&E: haematoxylin and eosin; M-T: Masson-Trichrome; IHC: immunocytochemistry; PAS: Periodic acid-Schiff; BEC: bronchial epithelial cells; RIA: radio-immunoassay; MMP: matrix metalloproteases; TIMP: tissue inhibitors of metalloproteases; VEGF: vascular epithelial growth factor; NA: not applicable; ?: unknown.

tissues provide a unique and global view of the pathological features of disease. In the same individual, the pulmonary tree can be studied from central airways to alveoli. Other components such as pulmonary circulation can also be studied. Specimens from autopsies have been instrumental in understanding the cause of fatal asthma [10]. The index designed by REID [61] and characterisation of emphysema subtypes [40, 43] have been established using surgically removed lungs from COPD patients. The discovery that remodelling also occurs in the distal airways and alveoli in asthma and COPD was based on studies using *post mortem* lung tissues. An increase in airway wall thickening and smooth muscle area was predominant in the distal airway of fatal asthmatics when compared with asthmatics who died from other causes [62]. Recently, the detection of chymase-positive mast cells in the distal airways has been suggested to be protective for lung function in severe asthma [63]. Surgical lung biopsies obtained by thoracotomy or thoracoscopy are the most invasive method to obtain lung/airway tissues. This procedure requires general anaesthesia and hospitalisation and includes a non-negligible risk of complications. For these reasons, surgical biopsies are not performed for research purposes. However, the unused lung specimen following pathological assessment for a medical condition can be used for research and many centres are now building tissue banks to collect, preserve and allocate these tissues for future research. Major limitations of *post mortem* and surgical tissues are that samples represent a severe stage of the disease, which has lead to death or surgery. There is also a less accurate knowledge of past medical history, an inability to perform physiological tests, the inapplicability to therapeutic studies and the presence of an underlying lung disease requiring surgical intervention (in most cases lung cancer). Nevertheless, these *post mortem* or surgical specimens allow a unique and overall understanding of the pathogenesis of airway diseases.

Endobronchial biopsies

Endobronchial biopsies (EBB) performed under flexible bronchoscopy are widely used in research today and are the standard approach to studying airway remodelling in diseases [64, 65]. Flexible bronchoscopy is a minimally invasive way to obtain central airway specimens. Histological analyses of these small pieces of airway tissue allow the identification of pathological changes amongst various diseases. EBB are also used to assess potential therapeutic effects in pharmacological studies. Technical and research aspects of EBB have been very well detailed in a recent publication by experts from the Endobronchial Biopsy Workshop [66]. In contrast to surgical tissues, EBB do not allow the study of the entire thickness of the airway and, therefore, results must be interpreted with this limitation. EBB allow the assessment of the remodelling to large airways only. Bronchoscopy units are accessible in most hospitals. EBB are easy to perform by well-trained respirologists, well tolerated by research subjects and can be repeated after challenge, treatment or during exacerbation. EBB have a significantly lower complication rate when compared with transbronchial biopsies.

Transbronchial biopsies

Transbronchial biopsies (TBB) are also performed under flexible bronchoscopy but sample the distal lung, including the distal airway wall and the alveolar tissues. Very few studies to date have been performed using transbronchial tissue [63, 67, 68]. The primary reason for limited studies is the concern about major complications, such as bleeding and pneumothorax. The pathogenesis of disease can be studied to a similar extent with TBB as with EBB; however, the success rate of obtaining a distal airway wall during TBB sampling is only ~30–50% per biopsy [66]. To bypass this low success rate, multiple biopsies need to be sampled, usually four to eight, but this is followed by an increased risk of complications. BALZAR *et al*. [69] demonstrated a similar inflammatory profile in the lungs of asthmatics using TBB and surgically obtained distal airways. Like EBB, TBB can be performed before and after treatment. Using TBB, the present authors recently demonstrated that remodelling can be assessed in distal airways of asthmatics and, furthermore, described the effect of inhaled corticosteroids (ICS) on inflammation and remodelling [67, 70]. TBB are an interesting tool to study distal airway involvement and therapeutic agents that target distal airways. The major limitations of TBB in airway remodelling research are the underlying risk of major complications related to the procedure itself and the difficulty in obtaining an adequate sample that contains distal airways.

Techniques for the assessment of remodelling in tissue

The standard assessment of remodelling is by histological examination of airway tissues. Tissue processing, visualisation

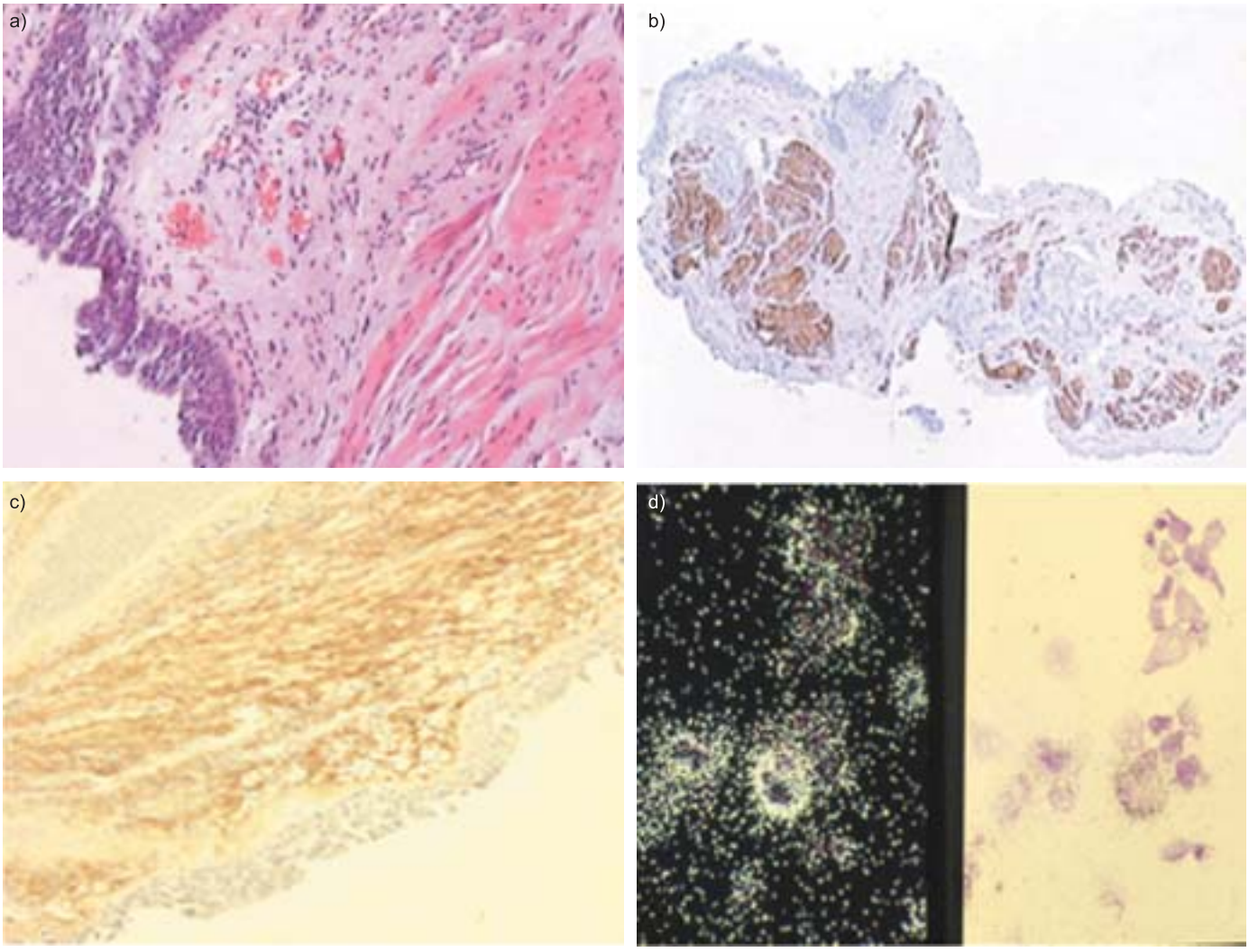

FIGURE 1. Examples of airway remodelling assessment in asthma and chronic obstructive pulmonary disease (COPD) using endobronchial biopsy tissue. a) Haematoxylin and eosin staining, allowing general morphology assessment of airway wall in COPD. b) Immunocytochemistry for smooth muscle α-actin (brown) in asthma. c) Immunocytochemistry for subepithelial collagen III fibres (brown) in asthma. d) *In situ* hybridisation for transforming growth factor (TGF)-β illustrating inflammatory cells expressing TGF-β mRNA; a positive signal appears bright white under dark field.

methods and quantification are all described in detail by JEFFERY *et al.* [66]. In brief, *post mortem* or surgical specimens, EBB and TBB need to be preserved following resection to allow future tissue analysis. Tissues are commonly preserved frozen or embedded in paraffin. Paraffin-embedded tissues show a better preservation of the airway wall morphology and are favoured for remodelling studies. Some remodelling measurements by electron microscopy have been proven to be very helpful in assessing the details of the subepithelial fibrosis and smooth muscle hyperplasia [24]. Airway remodelling is evaluated after histochemical (fig. 1a) and immunohistochemical (fig. 1b and c) staining. In general, the assessment of tissue structure is performed with haematoxylin and eosin staining on paraffin or frozen tissues. Sirius red, van Gieson or Masson-Trichrome stain the total collagen. Periodic-acid shiff staining is used to visualise the mucus glands. Immunohistochemistry allows detection of specific proteins thought to be involved in remodelling in the tissues such as ECM proteins [13, 23]. Moreover, specific antibodies directed against profibrotic cytokines can also be used. After staining, the slides are analysed under light microscopy. The results are usually expressed as the number of positive cells per area or the area occupied by the specific ECM component (or other) on the total area of the biopsy. The signal detected by immunohistochemistry reflects the tissue expression of proteins. mRNA expression of profibrotic cytokines can also be detected in tissues by *in situ* hybridisation. (fig. 1d). mRNA can be extracted from total tissues or from specific tissue areas by laser microdissection and then processed to study the expression of gene related to remodelling through Northern blot, real-time PCR following reverse transcription of mRNA or microarrays.

Indirect assessment of remodelling: body fluids

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is performed under flexible bronchoscopy and can be obtained at the same time as EBB.

BAL is routinely used to study cellular composition and to measure the levels of cytokines/chemokines in the distal airway and alveoli. However, individual components of the distal airway and alveolar compartments cannot be differentiated. The cellular component mainly represents the luminal inflammatory cells and some bronchial epithelial cells. An excess of bronchial epithelial cells in BAL is suggestive of epithelial fragility as commonly observed in asthma. Such analysis of the BAL content during health and disease or before and after treatment/challenge can compliment airway wall analysis. In fact, some inflammatory cells, such as neutrophils, are found more easily in the airway lumen whereas others, like lymphocytes, are mostly identified in the tissue. As airway remodelling occurs in the tissue, BAL analysis is not the optimal tool with which to study remodelling but serves only as an indirect marker of the remodelling and inflammatory processes that may be going on. Many mediators, including procollagen degradation and synthesis products, matrix metalloproteases (MMP) and tissue inhibitor of metalloproteases (TIMP) as well as profibrotic cytokines, can be quantified in BAL. MMP-9 and TIMP-1 have successfully been quantified and their activity assessed in BAL fluid of asthmatic subjects [71]. Elevated MMP-9 activity is detected in severe asthmatics and asthmatics with mucus hypersecretion, which may suggest a higher turnover of the ECM in the injured airways. In comparison to TBB, BAL fluid sampling is a low-risk procedure to study the distal airway and is well tolerated by subjects with minor complications such as mild fever. The limitations of studying airway remodelling using BAL fluid are: 1) the lack of discrimination between the distal airways and the alveoli; 2) both indirect and limited assessment of remodelling to some soluble markers related to ECM production and degradation; and 3) the inherent inconsistency in the withdrawn BAL volume and also the dilution factor.

Induced sputum

Hypertonic saline-induced sputum is a relatively noninvasive and easy technique to perform. It is commonly used to evaluate airway inflammation in the central airway. Soluble remodelling-associated proteins, such as procollagen synthesis peptides, MMPs, TIMPs and cytokines, can be detected in the sputum supernatants. Furthermore, induced sputum samples can be obtained before and after specific allergen challenge and/or therapeutic trial. Matrix degradation enzymes and inhibitors have been measured in the induced sputum, such as MMP-2, MMP-9, TIMP-1, TIMP-2, elastase and α_1-antitrypsin [72–75]. MMP-9 in induced sputum is elevated in severe asthmatics after allergen challenge and is not affected by ICS treatment [75]. This imbalance in the MMP-9/TIMP-1 ratio leads to excessive degradation of ECM proteins that participate in the injury–repair process. The turnover of collagen I can also be indirectly detected in sputum supernatant through the quantification of procollagen type I C-terminal peptide (PICP) and collagen type I C-terminal telopeptide (ICTP). PICP is representative of synthesis of collagen while ICTP reflects collagen degradation. It has been previously reported that PICP in sputum is increased during asthma exacerbation and is correlated with sputum eosinophils [76]. Angiogenic factor, vascular endothelial growth factor (VEGF) and anti-angiogenic endostatin were measured in the sputum supernatants of asthmatics [77], VEGF was found to be elevated in asthmatic sputum suggesting angiogenesis. Sputum processing requires the addition of a reducing mucolytic agent to facilitate the release of the cellular components from the mucus by protein denaturation. However, this significantly reduces the concentration of inflammatory cytokines in the sputum and directly interferes with the immunoassay. This is one of the major limitations in studying mediators in induced sputum. To avoid this limitation, fresh sputum samples without denaturation treatment must be used. Other limitations include the inconsistency of the dilution factor and the yield of obtaining an adequate specimen. However, it is a relatively noninvasive technique, well tolerated and easy to perform, which requires minimal equipment and staff. Sputum induction can also be performed repeatedly, before and after challenge/treatment, and even in children and exacerbated patients. For these reasons, the assessment of airway diseases through induced sputum analyses is gaining popularity in research.

Exhaled breath condensate

Exhaled breath condensate (EBC) is a noninvasive method of studying airway remodelling and reflects the composition of the fluid lining the airway. Several markers, including hydrogen peroxide, LTs, prostaglandins, isoprostanes, nitric oxide-derived products and hydrogen ions, have been measured successfully in EBC [78]. Such markers mainly reflect the inflammatory status of the airway and have been studied in patients with asthma, COPD or CF [79, 80]. However, the measurement of mediators within EBC is not very reproducible and the technique needs to be improved [81]. To date, no assessment of airway remodelling has been performed with EBC.

Blood

Markers of collagen synthesis and degradation can be quantified in blood. Amino-terminal propeptide of type III procollagen and ICTP have been measured in murine blood with liver fibrosis [82], suggesting that such markers can effectively be detected in blood. Other markers such as MMP-9, TIMP-1, cytokines, eotaxin and eosinophil cationic-protein have been measured in the plasma of asthmatics [83, 84]. Increased production and activity of MMP-9 have been reported in the serum of patients with severe asthma, following acute exacerbation. Raised MMP-9 levels in the serum have been proposed as an effective, noninvasive systemic marker of inflammation and airway remodelling in these patients [83, 84]. Serum TIMP-1 in COPD negatively correlates with airflow obstruction and exacerbation [85]. A low serum MMP-9/TIMP-1 ratio predicts the incomplete reversibility of forced expiratory volume in one second (FEV_1) to oral corticosteroids in asthma [86]. Blood sampling is a minimally invasive technique, easy to perform and may be repeated before and after treatment or challenge. However, the assessment of remodelling in blood is not specific to airways and blood markers from other organs can confuse measurements. Furthermore, the absence of a measurable amount of remodelling associated mediators cannot exclude their contribution as most of these proteins are synthesised locally in the lung and are not released in high enough quantities to be measured in the blood.

Urine

Urine sampling is not a common way to evaluate airway remodelling. In liver disease, the urinary concentration of the

collagen crosslink, pyridinoline and elastin correlated with liver fibrosis score in biopsy specimens from subjects with cirrhosis [82]. One recent study detected glycosaminoglycans (GAGs), a component of the ECM in asthmatic urine samples [87]. The level of GAGs was reduced after long-term treatment with ICS. Although not specific to airway remodelling, detection of airway remodelling markers in the urine might be an interesting avenue to explore.

Technique for assessment of remodelling in body fluids

Assessment of remodelling in fluids is quite different to tissues. Specific antibodies against collagens or proteases and anti-proteases are also used for protein quantification in fluids. Collagen degradation and synthesis products or MMP and TIMP can be quantified by an enzyme linked-immunosorbent assay or radio-immunoassay. If protein is abundant, Western blot analysis and zymography can be performed. All these techniques detect the protein level in fluids. Inflammatory cells can be assessed by immunocytochemistry and *in situ* hybridisation for the expression of profibrotic cytokines. mRNA can be extracted from inflammatory cells and then processed to study the gene expression through Northern blot, real-time PCR following reverse transcription of mRNA and microarrays.

Radiological assessment of airway remodelling

High-resolution computed tomography

It is also possible to assess the airway remodelling by high-resolution computed tomography (HRCT) of the thorax. This technique allows the study of the airway lumen and wall dimensions without invasive techniques, which might be useful to grossly assess remodelling in children [88] and in clinical trials [89]. AWADH *et al.* [90] reported a greater wall thickness with increased asthma severity. Asthmatics with incomplete reversible obstruction have an increased wall thickness, as demonstrated by HRCT, when compared with asthmatics with reversible airway obstruction [91]. However, the observations gathered from HRCT are not as detailed or as informative as histological examination of the tissue and unfortunately there are no studies to date comparing histological and radiological examination of the airways.

Endobronchial ultrasound

Over the last few years, endobronchial ultrasound (EBUS) has been developed, mainly to allow clinical diagnosis of bronchial carcinoma and targeting nodes sampling in lung cancer staging. However, in 2003, a case report described the measurement of subepithelial thickness/oedema by EBUS in asthmatics in which oedema was reduced by montelukast treatment [92]. One study has validated the measurement of thickening of the airway wall by EBUS, which correlated well to HRCT measurement [93]. Furthermore, a recent study in lung transplant recipients demonstrated that EBUS can discriminate different airway layers and help in the diagnosis of acute lung rejection [94]. Although exciting, EBUS is a relatively invasive technique and histological correlations are actually lacking. Up to now, EBUS cannot discriminate for the different components of the airway wall, such as smooth muscle bundles, and, therefore, the utility of EBUS is limited in airway remodelling research.

Physiological assessment of airway remodelling

Lung function data has been used to represent airway remodelling. In a longitudinal study from childhood to adulthood, a low post-bronchodilator FEV_1/vital capacity (VC) ratio was associated with an accelerated decline in lung function and reduced airway reversibility [95]. This study suggested that a low post-bronchodilator FEV_1/VC ratio is representative of airway remodelling and, importantly, that the remodelling process begins early in childhood. Reduced airway distensibility, measured by the change in anatomic dead space with lung volume, has been observed in asthmatics and proposed to reflect airway remodelling [96–98]. This hypothesis was confirmed by one study in which a negative correlation was observed between airway distensibility and reticular basement membrane (RBM) thickness in asthma [99]. From this study, change in dead space with lung volume was reflective of RBM thickness but not other remodelling features. More research needs to be carried out to study the sensitivity of the airway distensibility measurement in asthma severity and treatment response. Airway remodelling has been inconsistently correlated to lung function impairment, but studies using only lung function to assess airway remodelling should be taken with caution.

In vitro *remodelling models*

Primary airway structural cells, such as epithelial cells, fibroblasts and smooth muscle cells, can be successfully isolated from human airways. Thus, it is possible to study, *in vitro,* the fibrogenic and proliferative properties of individual cells following a wide range of stimuli. Recently, researchers have successfully been able to reconstruct airways *in vitro* using tissue engineering [100]. Such models can be used to study the interaction between structural cells and their response to injury. These reconstructed airways can be physically or chemically injured to study their *in vitro* repair process.

In summary, airway remodelling is clinically defined as persistent airflow obstruction despite aggressive anti-inflammatory therapies. The standard assessment of remodelling is obtained by surgical lung or airway specimens sampled through flexible bronchoscopy. Flexible bronchoscopy is a minimally invasive technique but requires specialist expertise. Tools have been developed to bypass the biopsy sampling. With indirect analysis of blood, urine or sputum remodelling markers, an insight is gained into the ongoing fibrotic process; however, it is not known whether the fluid variations in collagen products or proteases have significant consequences in the diseased airway walls. These alternative tools, including high-resolution computed tomography, endobronchial ultrasound, lung function measurement and tissue engineering, can be used as screening tools but modulation of airway remodelling will need to be confirmed in airway wall specimens.

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