The lower respiratory airway wall in children in health and disease

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Abstract

Alone or in association with other lung or thorax component disorders, the airway wall (AWW) remains one of the most frequently involved elements in paediatric lung diseases. A myriad of AWW disorders will present with similar symptomatology. It is thus important for the clinician to reappraise the normal development and structure of the AWW to better understand the underlying disease patterns. We herein provide an overview of the structure of the AWW and a description of its development from the fetal period to adulthood. We also detail the most common AWW changes observed in several acute and chronic respiratory disorders as well as after cigarette smoke or chronic pollution exposure. We then describe the relationship between the AWW structure and lung function. In addition, we present the different ways of investigating the AWW structure, from biopsies and histological analyses to the most recent noninvasive airway (AW) imaging techniques. Understanding the pathophysiological processes involved in an individual patient will lead to the judicious choice of nonspecific or specific personalised treatments, in order to prevent irreversible AW damage.

Lower airway wall structure

The lower airway (AW) (trachea, bronchi and terminal bronchioles) constitutes the conduction zone of the respiratory system. It ensures the passage of air from the upper AW to the respiratory bronchioles and alveoli where gaseous exchange takes place. From the lumen outwards, the airway wall (AWW) can be divided into four layers, each with specific functions: mucosa and submucosa (warming, moistening and removal of particulate materials and protective mechanisms against the external environment), musculo-cartilaginous layer (maintenance of the appropriate diameter of the AW), and adventitia (binding of the AW to adjacent structures such as the pulmonary artery and lung parenchyma). The posterior membranous portion of the trachea is only supported by a thin band of smooth muscle (the trachealis muscle, anterior to the oesophagus) whereas the anterior trachea is made up of C-shaped cartilaginous rings linked by annular fibro-connective tissue. Of note, cartilage is no longer present in the terminal bronchioles. The bronchial structure is very similar to that of the trachea, although in the main bronchi, cartilage completely encircles the lumen, whereas in the more distal bronchi cartilage is noncontinuous, in crescent shapes.

Maturational changes

In utero

The trachea and all bronchi are already formed by 16 weeks of gestation (figure 1). After this time, further growth occurs by elongation and widening of the AW. Epithelial differentiation occurs in parallel: epithelial thickness steadily decreases from early fetal to postnatal life [1]. The main epithelial cells (ciliated, basal and secretory goblet cells in proximal AWs and secretory club cells in distal bronchiolar
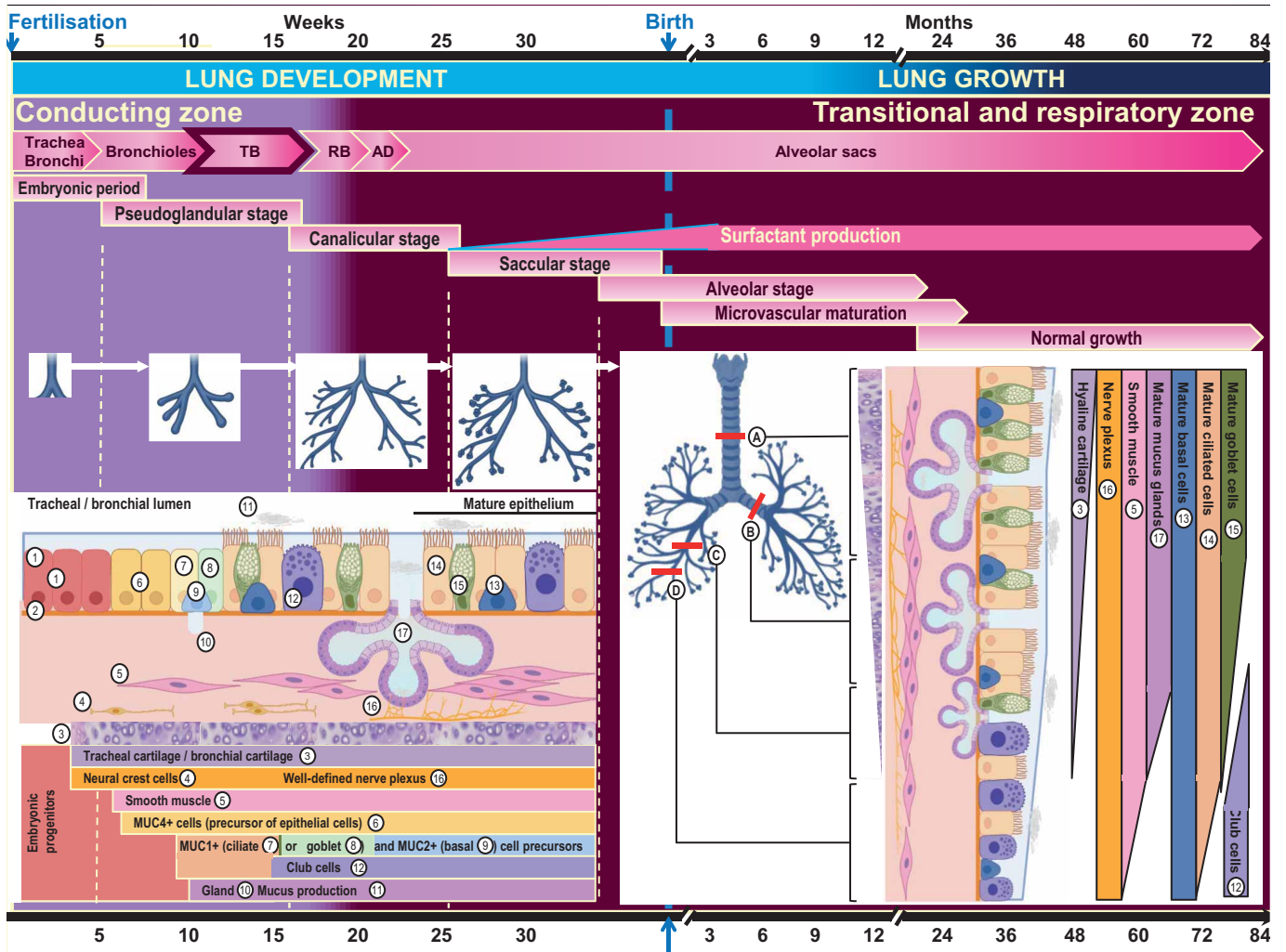
FIGURE 1 Changes occurring during the different phases of airway (AW) development. Bottom left: The numbers within a circle each represent a cell/structure type: 1: embryonic cell; 2: reticular basement membrane; 3: hyaline cartilage; 4: neural crest cell; 5: smooth muscle; 6: epithelial cell; 7: epithelial cell: future ciliated cell; 8: epithelial cell: future goblet cell; 9: epithelial cell: future basal cell; 10: glandular development: acellular bud; 11: mucus; 12: club cells; 13: basal cells; 14: mature ciliated cells; 15: mature goblet cells; 16: nerve plexus; 17: mature mucus gland. Bottom right: This indicates cell/tissue type according to AW generations. TB: terminal bronchioles; RB: respiratory bronchioles; AD: alveolar ducts.

AWs) all derive from a common pluripotent embryonic epithelium. The tracheobronchial epithelium remains undifferentiated until 10 weeks of gestation, and then glandular development begins, creating an acellular bud which grows into the mesenchyma and then forms a lumen.

Apart from their protective role, mucus-secreting cells act as stem cells during fetal development [2]. Several mucin (MUC) genes encoding the protein core of mucin have been identified early during fetal AW development. MUC4 is the earliest gene expressed in the foregut (6.5 weeks of gestation), followed by MUC1 and MUC2 from 9.5 weeks in the trachea, bronchi and epithelial tubules [3]. MUC4 and MUC1 mRNAs are located in goblet and ciliated cells, whereas MUC2 mRNAs are located in basal and goblet cells [3]. This differential expression suggests distinct developmental roles for MUC1, MUC2, MUC4, MUC5AC, MUC5B and MUC7 in the elongation, branching and epithelial cytodifferentiation of the respiratory tract during ontogenesis. MUC1, MUC2, MUC3, MUC7 and MUC8 may also contribute to the biosynthesis of AW mucus, whose secretion begins by 13 weeks of gestation. Interestingly, the increased number of AW secretory cells and chemical composition of their secretions during the second trimester show similarities with what is observed in some disorders such as chronic bronchitis, bronchiectasis, asthma and cystic fibrosis (CF) [2, 3]. However, the biological role of mucus *in utero* is not clear, but it may protect the AW epithelium against proteolysis [4].

By this time cilia appear in the proximal AW [5]. Club cells appear by 15 weeks of gestation in human bronchioles [6]. The differentiation of ciliated and secretory cells increases progressively up to 18 weeks of gestation. A mature and functional mucociliary epithelium is established by 23 weeks of gestation (including goblet cells) [1]. As an integral part of the branching epithelial tubules, a coat of airway smooth muscle (ASM) and an abundant neural plexus ensheathing the AWs develop very early [7]. At 6–8 weeks of gestation, ASM cells appear in the trachea and bronchi. Muscle then develops sequentially and peripherally. During fetal life and childhood there is an increase in the amount of ASM relative to the size of the AWs. The ASM at birth has a mature structure, is innervated, and has been shown to contract [1]. Cartilage first appears in the 4th gestational week in the trachea, the 10th week in the main bronchi, and the 12th week in segmental bronchi [1]. Neural crest cells appear in the future trachea at 4–5 weeks of gestation. The anatomical features of the sympathetic–parasympathetic systems are established by 6 weeks; by 16 weeks, there is a well-defined posterior plexus and an inner plexus involving the cartilage, epithelium, submucosal glands and tracheal muscle [1].

Postnatal

Postnatally, alveoli continue to develop and AWs continue to elongate. Rapid alveolar multiplication occurs until the end of 2 years [8]. Thereafter, alveolarisation continues until adult age: the number of alveoli is estimated to increase 1.94-fold (95% CI 1.64–2.30) from the age of 7 to 21 years [9]. This is accompanied by rapid pubertal three-dimensional enlargement [8, 10].

There are intrinsic differences in regenerative capacity and transcriptional profile between proximal AW epithelial cells of children *versus* adults [11], despite the fact that the cellular composition of the tracheobronchial epithelium is similar in both age groups. In cell culture, paediatric AW epithelial cells displayed more robust growth. In bulk RNA sequencing experiments from tracheal biopsies, pathways such as TNFα (tumour necrosis factor), MTORC1 (mammalian target of rapamycin complex 1) signalling, and processes such as inflammation and apoptosis, were more marked in paediatric basal cells [11]. In contrast, in cultured basal cells, the mucins *MUC2*, *MUC3A*, *MUC5AC*, *MUC5B* and *MUC17*, as well as the secretory master regulator *SPDEF* (SAM pointed domain-containing Ets transcription factor), were more expressed in adults *versus* children [11].

Few new AW submucosal glands are synthesised after birth. An increase in gland area with age mostly results from increased gland complexity [1].

The growth of the AW occurs more slowly than that of lung parenchyma, particularly in the first years of life [8, 12]. This is known as disynaptic growth, *i.e.* disproportionate but physiologically normal lung growth, responsible for biological variability regarding AW size compared with lung size between subjects of similar height and gender. It is noteworthy that during infancy, girls have proportionally larger AWs relative to lung size than boys [8]. A greater number of alveoli in males will limit AW growth (AWs can only increase their dimensions according to alveolar growth because they are surrounded by them). This would suggest that development of their peripheral and bronchial AW dimensions relative to the growth of lung volume is delayed (mainly after the age of 4–5 years) *versus* girls [8]. Although a physiological phenomenon, disynaptic growth patterns of the AW relative to the lung parenchyma might predispose to AW disease.

Approximately 3–5% of the mainstem bronchial wall is occupied by ASM in children and adults, whereas in bronchioli it is 10–20% in the former and 20% in the latter. There is a more rapid increase in the amount of ASM immediately after birth [1, 13]. Compared to adults, stimulated neonatal human ASMC (ASM cells) are in a rapid and robust proliferative phase and have the capacity to respond disproportionately under abnormal environmental conditions, through increased mitochondrial biogenesis and altered calcium homeostasis [14]. ASM contributes by multiple mechanisms to the natural hyperresponsiveness of the young [15]. AW contractile force was 25–100% greater in 2-week-old farm swine compared to 10-week-old swine. Differences in force generation were not related to morphometric ASM receptor distribution, morphological changes in AW muscle mass, cellularity, changes in content of nonmyocyte tissues, or tissue content of functional myosin isoform [16]. Tracheal strips from adult guinea-pigs relax to a level of tension similar to that found in the absence of stimulation; this ability to spontaneously relax is essentially absent in trachealis muscle from infant animals [17]. Overall, there is evidence: 1) for a natural decline after newborn and juvenile life in the shortening velocity of ASM; 2) impaired relaxation related to changes in prostaglandin synthesis and acetylcholinesterase function; 3) a dynamic role for the cytoskeleton in facilitating and opposing ASM shortening (oscillatory strain relaxes adult ASM, but potentiates active stress in newborns) [17].

A balance of AW rigidity and elasticity is critical for uninterrupted airflow to and from the lungs. In the trachea and bronchi, this balance is achieved by the precise juxtaposition of ASM and cartilage that together encircle the AW [18]. Cartilage continues to form peripherally until a postnatal age of about 2 months. Thereafter, there is little further extension but there is a progressive increase in the total cartilage mass throughout childhood. At birth the distribution and number of nerves to all AW structures is similar to that in the adult, with sympathetic and parasympathetic nerve fibres extending as far as the alveolar ducts [7]. Functional studies in humans suggest an increase in β-adrenoreceptors and a decrease in muscarinic receptors with age [19].

The observable alterations in the AWW in paediatric respiratory disorders are described in Supplementary material 1. Figure 2 also indicates the onset and the close relationship between these AW diseases, from infancy to adolescence. The end result is paediatric chronic suppurative and nonsuppurative obstructive airway diseases (pCOAD), whose roots occur during childhood but which manifest further during adulthood.

Structure–function of lower AWs

Maturational changes in bronchial wall compliance have not been extensively studied. In pigs, semistatic pressure–volume curves in isolated bronchi showed that the specific compliance halved from 1 to 4 weeks of age. No change was observed either between 4-week-old and adult pigs [20]. Changes in the total wall and cartilage areas did not correlate with changes in specific compliance. Inflation to 20 cmH_2O transmural pressure reduced the total wall area of bronchi from 1-week-old pigs [20]. The increase in luminal volume during inflation of bronchial segments occurs, partially, by compression of the AWW against the cartilage layer. Thus, both the specific compliance and the maximum inflation of isolated bronchi decrease with maturation. Similarly, human tracheal compliance decreases progressively from infancy to adulthood. Infant bronchi are approximately twice as compliant as those in 4–11-year-old children [20, 21]. Morphometric assessment of ASM length showed greater muscle shortening to acetylcholine in cartilage-removed AW than in controls [22]. AW narrowing was positively correlated with AW compliance. Compliance and area of cartilage were negatively correlated. Thus, AW narrowing is increased in compliant AW and cartilage significantly loads ASM in whole bronchi [22].

Relating the AWs to the whole lung (AW–parenchymal interdependence)

The pathophysiology of obstructive disease relies on the factors that determine the calibre of an individual AW, *i.e.* the force balance between 1) the inward elastic recoil of the AWW, 2) the outward tethering forces of its parenchymal attachments (the outsides of the walls of the intrapulmonary AW, embedded in the lung parenchyma, are attached to alveolar tissue that is under tension; the parenchyma exerts an outward tethering force on the AW that increases as lung volume increases), and 3) any additional forces due to contraction of ASM. Other factors contribute to AW narrowing, such as 4) thickening of the AWW and 5) accumulation of secretions in the lumen. AW obstruction becomes particularly severe when these various factors occur simultaneously. However, the effect of AW abnormalities on lung function cannot be fully understood only in terms of what happens to a single AW because narrowing throughout the AW tree is heterogeneous and interdependent. The AW tree undergoes an enormous amplification in cross-sectional area from the proximal to the very distal AW. Obstructive lung pathologies thus depend on the way in which the AW tree behaves as a system [23].

The ability of a branch of the AW tree to transmit gas flow is essentially a function of its length and diameter. During quiet breathing, airflow through most branches of the AW tree is laminar and steady; so the Poiseuille formula ($R = 8.\mu.l / \pi.r^4$) provides a useful approximation to the resistance, R, of a branch of length l and radius r (where μ = gas viscosity). The most potent geometric determinant of R is r, so a measurement of the resistance of the AW tree, which is a reflection of function, provides a direct link to the calibre of the AW, which is a reflection of structure [23].

Computational fluid dynamics modelling of respiratory airflow dynamics in the AW generations G6–G9 of infants, children and adults show that velocity, pressure and wall shear stress decrease with age during inspiration and expiration. The influences of the respiratory mechanics on the airflow in the age groups were reflected in the distribution of the velocity streamlines (the splitting of velocity streamlines at bifurcations increases during inspiration with age) [24].

The AWW also contributes towards obstruction by secreting mucus, cells, fluid, *etc.* into the AW lumen. Excessive dysfunctional mucus participates in the pathogenesis of all the common AW diseases [25]. In CF, for example, dramatic increases in dynamic viscosity and elasticity have been reported during a pulmonary exacerbation with return to baseline upon recovery. Sputum viscoelastic properties thus correlate with lung function and disease status [26].
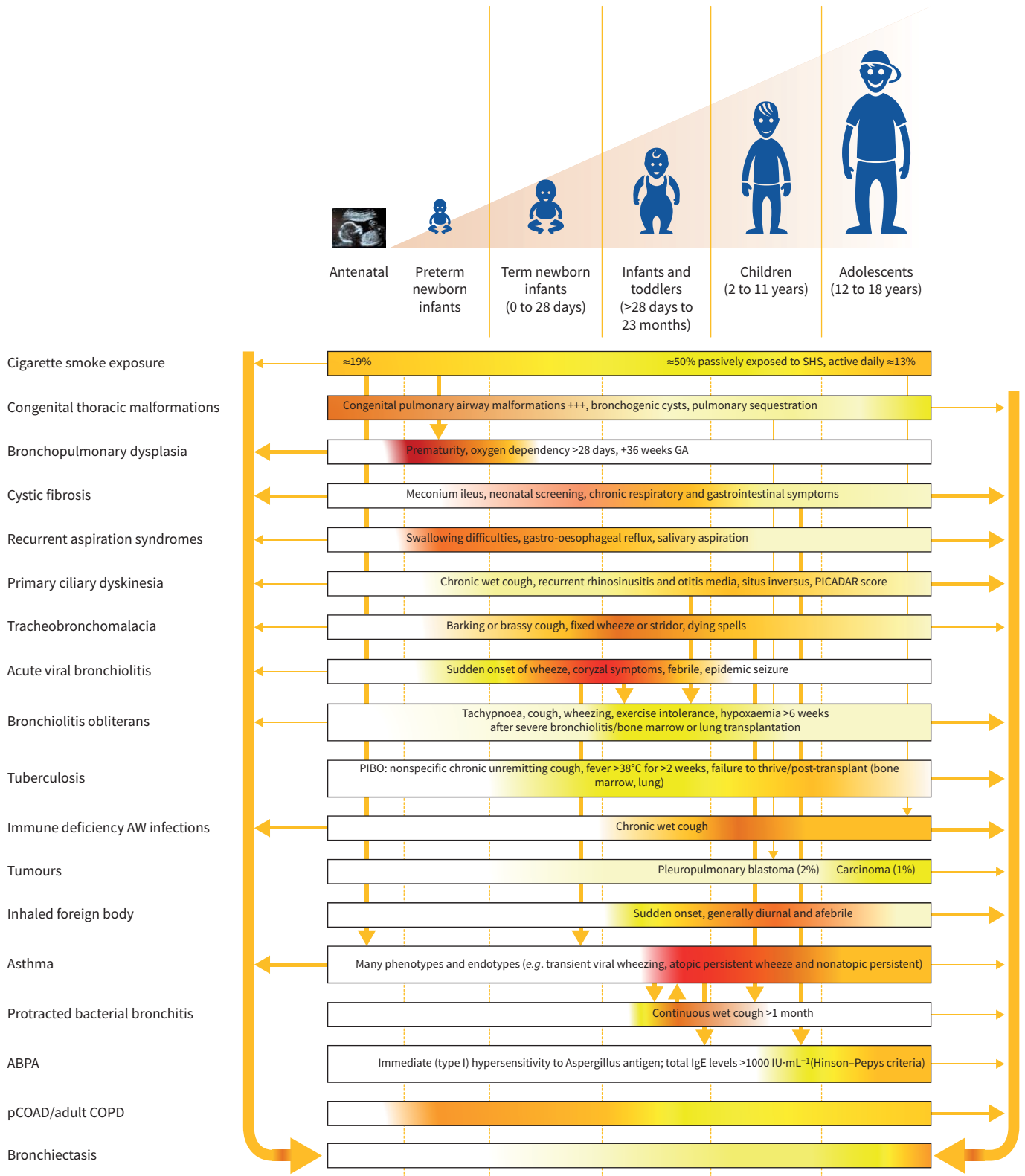
FIGURE 2 Relationship between airways (AWs) from infancy to adolescence. The arrows indicate the links between the AW diseases. The colour code represents a frequency "heat map": white indicates no risk; yellow to red indicates increasing risk. ABPA: allergic bronchopulmonary aspergillosis; pCOAD: paediatric chronic (suppurative and nonsuppurative) obstructive airway disease; GA: gestational age; PIBO: post-infectious bronchiolitis obliterans; SHS: second-hand smoking.

Role of deep inspiration (DI)

AW–parenchymal interdependence may contribute to the phenomenon known as mechanically-induced bronchoprotection, by which one or more DIs taken before administering a bronchoconstricting agent attenuates the bronchoconstricting response [27]. Refraining from taking regular DIs increases AW responsiveness in normal and asthmatic subjects when the response is measured by a fall in FEV_1. Avoiding DI increases the maximal response to methacholine without altering sensitivity in nonasthmatics. The bronchoprotective effect of DI is lost even in people who have mild asthma and obese subjects (decreased lung volumes in the latter). This has also been shown in school-age children, suggesting that the forces that determine AW calibre in such children are similar to those in adults [28]. One possible explanation for this phenomenon is through its effect on the plasticity (adaptability) of ASM. ASM becomes stiffer if not subjected to periodic strain [29], and its contractile capacity is temporarily lessened after an acute length change, as occurs during DI. Interestingly, the bronchoprotection of DI attenuates the decline in FEV_1, but does not affect the increase in AW resistance (R_{aw}), following bronchoprovocation. AW hyperresponsiveness in asthma may not be a problem of too much ASM strength. Rather, it may be a problem of too little of the factors that oppose muscle shortening. Loss of the dilating response to a DI may play a central role in this process, involving the ASM (bridge dynamics, plastic reorganisation of the cytoskeleton) [30]. Another possibility resides in alveolar attachment–AWW interdependence: in COPD, it has been shown that the decrease in AWW stretch due to loss of alveolar attachments contributes to the loss of the bronchodilatory effect of lung inflation [31].

Clinical signs of AW disease

The clinical findings that point to the lower respiratory tract include inspiratory and expiratory ronchi, wheeze and expiratory stridor for the large AW, and coarse crackles (heard on early inspiration and throughout expiration) and squawks for the distal AW [32]. Wheezes may be absent if airflow is too low (*e.g.* in severe asthma). These clinical signs are nonspecific. Localised decreased lung sounds may indicate AW conditions such as an endobronchial foreign body or tumour [32].

Investigations

Open lung biopsy or bronchial mucosal biopsies taken during bronchoscopy are the gold standards to visualise and measure the different AWW layers very precisely, but are invasive [33, 34]. These biopsies provide information from small selected sites of the AW and are subject to artefacts. However, this technique is a huge addition to the understanding of the pathophysiology of AW diseases in childhood, in particular asthma, CF and bronchiectasis [35].

Bronchoscopy remains one of the main investigations in all AW diseases. More recent additions to the technique have been proposed. In adults, AWW structure can be assessed during bronchoscopy, without biopsy, by endobronchial ultrasonography (EBUS) [36]. The thickness of the whole AW, especially the submucosal layer containing ASM, is significantly greater in patients with asthma than controls. Furthermore, bronchial responsiveness is negatively correlated with the thickness of the second layer measured by EBUS. Alternatively, optical coherence tomography (OCT), generated from near-infrared light cross-sectional images created by the backscattering of light by the tissue provides highly detailed images of the AWW. A significant correlation was found between *ex vivo* OCT imaging and histology with minimal bias in the Bland–Altman analysis, and high interobserver reproducibility and intra-class correlation [37]. These techniques are considered to be accurate and reproducible for the identification and quantification of AWW layers, and hold promise to identify and quantify AW remodelling in obstructive lung diseases.

AW imaging
Chest X-ray

Findings of chronic AW disease are nonspecific and include increased bronchovascular markings. Widespread small AW involvement is needed before abnormalities become apparent [38]. It is of utmost importance to look for evidence of the consequences of AW obstruction such as unilateral hyperinflation, atelectasis or mediastinal shift, on inspiratory and also on expiratory views if required. In an acute setting the presence of cough, fever and wheeze with radiological bronchial wall thickening with air trapping in a child younger than 2 years old suggests a diagnosis of acute viral bronchiolitis. In an older child, the same combination of findings indicates the presence of acute asthma, whereas the absence of air trapping suggests lower respiratory tract infections [39]. The injection of contrast medium into the AW (bronchography) to diagnose structural or functional abnormalities is considered obsolete. However, contrast medium (barium, gastrografin, *etc.*) opacification studies *via* the oesophagus (swallow studies) are still commonly performed to detect trachea-oesophageal fistulas.

CT scan is a useful tool to noninvasively assess AW remodelling

Quantitative AW measurements from chest CT are feasible and correlate with pulmonary function in paediatric post-infectious bronchiolitis obliterans in children as young as 4 years of age [40]. In CF, AW/artery thickness has been used to compare bronchiectasis scores in children aged 6 years and over [41]. The diagnosis of bronchiectasis is highly dependent on lung volume and more reliably diagnosed using outer AW diameter [42]. AW remodelling (AWW thickening) in asthma can be assessed by high-resolution computed tomography scan of the chest in adults [43, 44], school age [45, 46] and preschool [47] children. Interestingly, CT total AW count was significantly diminished in adults with greater asthma severity and was related to AWW thickness and MRI ventilation defects [48]. A greater number of missing sub-subsegmental AWs was associated with thicker AWW and narrower AW lumens. Intraluminal occlusions did not have a large impact on total airway count (TAC), and it was suggested that it is obstruction (*via* AWW remodelling or collapse) rather than AW destruction that is responsible for reduced TAC in severe asthma.

High-quality MRI is feasible and increasingly practical in the evaluation of a number of diseases involving the paediatric large AW. Studies have shown that with attention to patient preparation, protocol optimisation and appropriate use of sedation, excellent MRI can be performed for various clinical indications [49]. Smaller paediatric AWs have also been studied by MRI [50]. In children with CF aged 9 years and older, submillimetre spatial resolution of T1-weighted pointwise encoding time reduction with radial acquisition (PETRA) imaging showed a clear delineation of AWW and lumen, including low-grade modifications [51, 52]. Regarding bronchiectasis, the agreement between chest CT and MRI PETRA sequences was good (Kappa score 0.83 [95% CI 0.78–0.87]) [51, 52]. In younger sedated preschool children with stable CF, MRI (including bronchial wall abnormalities: wall thickening and/or bronchiectasis and mucus plugging) could be successfully performed with diagnostic quality in 100% of patients [53].

Lung function testing

Lung function testing only provides indirect information regarding the AWW (obstructive or mixed pattern, increased resistance, distension, AW hyperresponsiveness, bronchodilator reversibility, fixed obstruction). Spirometry is considered the main method to detect airflow limitation. Several studies have demonstrated a negative correlation between FEV_1 and CT scan bronchial wall thickening in PCD [54], CF [55] and asthma [56]. In childhood asthma, however, the correlation between FEV_1 and AWW thickening is more controversial and mostly depends on the severity of the disease. Indeed, in severe asthma in children, FEV_1 negatively correlated with ASM area ($r=-0.6$; $p=0.002$) [57]. Lung function testing cannot be used to diagnose tracheobronchomalacia but a fixed obstructive AW pattern (early phase plateauing of the expiratory limb; a plateau of both inspiratory and expiratory limbs of the flow volume loop) is supportive evidence [58]. Airflow limitation occurs in part due to increased AW resistance, which can be measured directly. AW resistance is determined by the diameter of the AW, the velocity of airflow and the physical properties of the gas breathed [59]. Although alterations in bronchomotor tone play a role, it is the decrease in lung elastic recoil as lung volume declines that is the predominant mechanism for the change in AW resistance [60]. AW resistance is traditionally measured by relating airflow and driving pressure using body plethysmography, but other methods including forced oscillation have been proposed. This technique requires minimal patient cooperation, but can be used to measure the effect of DIs. SCHWEITZER *et al.* [61] have shown that the response of respiratory resistance during inspiration to DI may prove useful in identifying the mechanism of AW obstruction (increased bronchial wall hysteresis, and therefore smooth muscle contraction, as a mechanism to exercise-induced bronchoconstriction). TIDDENS *et al.* [62] have measured the AW dimensions of tissue specimens from CF *versus* COPD patients and estimated the importance of these dimensions to AW resistance using a computational model. The inner wall and smooth muscle areas of peripheral CF AW were increased 3.3- and 4.3-fold, respectively, compared to those of COPD AW. The epithelium was 53% greater in height in peripheral CF AW. The sensitivity and maximal plateau resistance of the computed dose/response curves were substantially increased in the CF patients compared to COPD patients. Multiple breath nitrogen washout (MBNW) quantifies overall ventilation inhomogeneity (lung clearance index at 2.5% (1/40th) of the starting nitrogen concentration ($LCI_{2.5}$)). In nonsevere asthma $LCI_{2.5}$ is mostly within normal limits [63], in contrast to CF and PCD, and does not correlate with RBM thickness [64].

An important question is the contribution of AW hyperresponsiveness (AHR) towards our understanding of AW function for clinical use, especially in very young children. AHR results from a multitude of potential underlying mechanisms, which are likely to have different contributions amongst individuals. As stated above, ASM participation in AW obstruction can be explored by bronchial relaxation and provocation tests.

TABLE 1 Diagnostic methods and strategy for suspected AW diseases

Least invasive				Most invasive
Clinical indices	Lung function tests	Imaging	Not or minimally invasive biologic tests/procedures	More invasive procedures

• Prematurity
• Age at onset of symptoms
• Initial clinical picture
• Patient age
• Acute/chronic
• Time of the year
  - September (rhinovirus)[#]
  - End of year (RSV)[#]
  - Start and end of year (influenza)[#]
• Efficacy of past treatments
• Lung or bone marrow transplant
• Probability scores available
  - Colom and Teper (obliterative bronchiolitis)
  - PICADAR score (PCD)
• Algorithm available (PCD)
• Neonatal screening (CF)
• Therapeutic trial
  - Antibiotics (protracted bacterial bronchitis)
  - Regular asthma therapy (asthma)
  - Systemic steroids, long-term or IM (asthma with obstruction not reversible by SABA)
	• Spirometry (obstructive or mixed pattern)
	• Plethysmography, R_{aw} (suspected distension)
	• R_{rs}, R_{os}, R_{int} (↑)
	• F_{eNO}
	  - Lower AW ↑ (asthma)
	  - Nasal ↓ (PCD)
	• AW BHR
	• BD reversibility
	• Lung clearance index (CF, PCD)
		• Chest X-ray
		• Inspiratory and expiratory films (inhaled foreign body)
		• Contrast medium; videofluoroscopic swallowing study (tracheo-oesophageal fistula; chronic aspiration)
		• CT scan (various, severe disease, CF, non-CF bronchiectasis, TB)
		  - Mosaic pattern (obliterative bronchiolitis)
		• CT scan + virtual endoscopy (foreign body, stenosis, obstruction)
		• CT scan + contrast
		  - Vascular anomalies
		  - Haemoptysis (embolisation of bronchial or systemic arteries)
		• MRI (bronchiectasis, AW malformations, haemangiomas)
		• MRI + contrast (vascular abnormalities)
		• MRI + ventilation (hyperpolarised gas) and perfusion studies (CF, BPD)
		• Octreotide scans, PET scans (tumours; may be negative in carcinoids)
			• Blood
			  - RAST tests (allergy)
			  - Immune screen (bronchiectasis)
			  - Genetic tests (CF, PCD)
			  - Serology (infections)
			  - Cotinine
			• Sputum
			  - Microbiology (CF, TB)
			  - Inflammatory markers (asthma, CF)
			• Naso- and oro-pharyngeal swabs
			  - Virology
			  - *Mycoplasma pneumoniae*
			  - *Chlamydia pneumoniae*
			• Gastric aspirates
			• Nasal brushing (PCD)
			• Skin
			  - Skin prick and patch tests (allergy)
			  - Sweat test (CF)
			• Urinary cotinine
			• Hair cotinine
			• Saliva
			  - Cotinine
			  - Genetic tests
				• Bronchoscopy
				  - Direct vision (foreign body, AW malformations)
				  - EBUS (older children)
				  - Dynamic (tracheo-bronchomalacia)
				  - Swallow studies (aspiration syndromes)
				  - Bronchoalveolar lavage (asthma, bronchiectasis, CF, protracted bacterial bronchitis, chronic aspiration (lipid-laden macrophages))
				  - Lower AW brushings (PCD)
				  - Biopsy (asthma, TB), tumours (carcinoids: risk of bleeding)
				• Surgery (AW malformations, localised bronchiectasis)

[#]: in Europe (Northern Hemisphere). In brackets are indicated the diseases for which the clinical index/test/procedure is frequently concerned. AW: airway; RAST: radioallergosorbent test; EBUS: endobronchial ultrasonography; CF: cystic fibrosis; R_{aw}: airway resistance; R_{rs}: respiratory resistance; R_{os}: respiratory resistance measured by oscillometry; R_{int}: interrupter respiratory resistance; PCD: primary ciliary dyskinesia; F_{ENO}: fractional exhaled nitric oxide; CT: computed tomography; RSV: respiratory syncytial virus; TB: tuberculosis; BHR: bronchial hyperreactivity; BD: bronchodilator; MRI: magnetic resonance imaging; BPD: bronchopulmonary dysplasia; PET: positron emission tomography; IM: intramuscular; SABA: short-acting beta-agonist.

After preterm birth, AHR is related to low gestational age at delivery and a neonatal history of bronchopulmonary dysplasia. No studies have reported associations between AHR after preterm birth and the markers of eosinophilic inflammatory AW responses typically found in asthma [65, 66]. Preterm-born subjects, especially those who had chronic lung disease, had increased rates of bronchial hyperreactivity to direct (methacholine) and indirect (exercise) stimuli compared to term-born subjects [66].

It has been suggested that persistent AHR in asthma corresponds to a "Forever Young" [67] or "Regression Toward a Pre-natal" state [68]. In asthmatic infants, on the one hand AHR is predictive of subsequent clinical course (adenosine 5′-monophosphate (AMP) challenge using the auscultation method (AMP-PCW) [69] or methacholine challenge). Increased AHR to methacholine in symptomatic infants was associated

with increased AHR, doctor-diagnosed asthma and exercise-induced bronchospasm at the age of 6 years [70]. On the other hand, when children with asthma with exacerbations and those without exacerbations were followed from age 1 month until 13 years, it was found that AW obstruction and AHR were stable traits of childhood asthma since neonatal life, suggesting that symptomatic disease may in part be a consequence of these traits, but not their cause [71]. It is worth noting, however, that the prognostic value of AHR may depend on the challenge agent when assessing the prognostic value of AHR. Results from hypertonic saline challenge are associated with persistent asthma symptoms even after a decade [72], while AHR measured by indirect methods at preschool age did not predict AHR in adolescence [72]. The relationship between AWW thickness and AW responsiveness is also subject to discussion. Because AHR is believed to be a marker of asthma severity, AHR and wall thickness should be positively correlated. Individuals who have thicker AWs are more responsive [73], but this was not the case in all studies [74, 75]. In fact, these authors reported that AWW thickening attenuates AW reactivity to methacholine in patients with asthma [56, 74]. AHR has been used to guide asthma treatment in children [76]. In children aged 6–16 years with moderate atopic asthma, after 2 years of treatment no difference was found in the percentage of symptom-free days between treatment strategies (either based on symptom or methacholine challenge results). However, pre-bronchodilator FEV_1 was higher in the AHR strategy (2.3% predicted), this being entirely explained by worsening of FEV_1 in a subgroup of hyperresponsive children with low symptom scores. In the AHR strategy arm, 47% of decisions would have been different had patients been treated according to the reference strategy (higher medication level in 24%; lower level in 23%). The mean ±SEM daily inhaled corticosteroid (ICS) dose was 478±27 mg fluticasone in the reference strategy arm and 562±26 mg in the AHR arm. The largest difference between strategies was seen after 6 months in the AHR subgroup, but at the end of the study, treatment levels were similar in both groups [76].

In CF, reversible AW obstruction is common, being more frequent in younger patients and with severe genotype, with no correlation to markers of atopy or CF clinical severity [77].

Management

The early recognition of AW involvement is of capital importance because bronchiectasis, AW obliteration and/or remodelling are in most cases irreversible, despite medical intervention. Of note, although quite rare, early bronchiectasis may be reduced or even resolved, in particular in case of infections [78] or extracted foreign bodies [79]. The overall diagnostic methods available are described in table 1. We recommend an algorithmic symptom-based approach, as proposed in chronic cough [80, 81] or recurrent wheezing [82].

Conclusion

The components of the AWW are mature and functional at birth, while cartilage rigidity and AW dimensions increase with time. In disease conditions, histological changes in the AWW vary according to the underlying conditions. Modifications in all the layers of the AWW act concurrently to accentuate the impact of the AW calibre on lung function. Importantly, AW–parenchymal interaction and ASM characteristics and behaviour in health and disease also determine how gas is transmitted through these conducting AWs. Newer methods of investigation, in particular in the field of imaging, are essential for the understanding of diseases linked to modifications in the AWW.

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