Interstitial lung disease in infancy and early childhood: a clinicopathological primer

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ILD of childhood is comparable by name only to lung disease in adults. A dedicated interdisciplinary team is required to achieve the best possible outcome. This review summarises the current clinicopathological criteria and associated genetic alterations. https://bit.ly/3mpxI3b

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Abstract

Children's interstitial lung disease (chILD) encompasses a wide and heterogeneous spectrum of diseases substantially different from that of adults. Established classification systems divide chILD into conditions more prevalent in infancy and other conditions occurring at any age. This categorisation is based on a multidisciplinary approach including clinical, radiological, genetic and histological findings. The diagnostic evaluation may include lung biopsies if other diagnostic approaches failed to identify a precise chILD entity, or if severe or refractory respiratory distress of unknown cause is present. As the majority of children will be evaluated and diagnosed outside of specialist centres, this review summarises relevant clinical, genetic and histological findings of chILD to provide assistance in clinical assessment and rational diagnostics.

Introduction

Children's interstitial lung disease (chILD) encompasses a heterogeneous group of innate, genetic, infectious and inflammatory diseases, quite different from that seen in adulthood [1]. Since the prevalence of individual entities is likely <1 per 100 000 individuals, hospitals see so few cases that relevant experience regarding diagnosis and treatment is often limited [2, 3]. Helpful guidelines outlining clinical assessment, imaging and/or genetic as well as histological analyses to achieve a specific diagnosis have been established [2, 4]. The first classification system of interstitial lung disease (ILD) was developed for adult diffuse lung diseases and mainly based on histology [5]. Subsequently, a multidisciplinary approach resulted in improved standardised terminology and diagnostic criteria [6]. Direct application of the adult classification scheme to chILD is limited by a differing frequency and spectrum of disease as well as divergent clinical manifestations [7, 8]. For instance, the common adult histological pattern of usual interstitial pneumonia is rarely, if ever, seen in infancy or childhood. Moreover, morphological patterns occurring in both age groups often have divergent aetiological and prognostic implications. Thus, age-adapted classification systems, at first histology driven [7], later based on multidisciplinary diagnostics, had to be established for children [1, 3, 9–14]. All classification systems share a separation into chILD typical for early childhood and chILD without any specific relationship to age. Table 1 depicts the classification of chILD as proposed by the chILD-EU network [3]. This review summarises the classification and diagnostic criteria of ILD more prevalent in infancy, with special emphasis on genetics and histology.

TABLE 1 Classification of children's interstitial lung disease (chILD) (modified after [3, 7])

ILD: more prevalent in infancy	
A1: diffuse development disorders	Acinar dysplasia Alveolocapillary dysplasia with misalignment of pulmonary veins Congenital alveolar dysplasia
A2: growth abnormalities	Alveolar simplification Chronic neonatal lung disease Bronchopulmonary dysplasia Chromosomal alterations
A3: specific entities of undefined aetiology	Pulmonary interstitial glycogenosis Neuroendocrine hyperplasia of infancy
A4: Surfactant dysfunction mutations and related disorders	Pulmonary alveolar proteinosis Chronic pneumonitis of infancy Desquamative interstitial pneumonia Nonspecific interstitial pneumonia
Ax: unclear respiratory distress syndrome in the mature neonate	
Ay: unclear respiratory distress syndrome in the almost mature neonate (30–36 weeks)	
ILD: not specific to infancy and childhood	
B1: ILD related to systemic disease processes	Storage disease Langerhans cell histiocytosis Endogenous lipid pneumonia Immune-related disorders
B2: ILD of the normal host and due to exposures	Hypersensitivity pneumonitis Infection Aspiration pneumonia Eosinophilic bronchiolitis
B3: ILD of the immunocompromised host	Infection Obliterative bronchiolitis/restrictive allograft syndrome
B4: ILD with structural vascular changes	Pulmonary hypertension Pulmonary veno-occlusive disease Pulmonary capillary haemangiomatosis Vasculitis
B5: ILD related to reactive lymphoid lesions	Follicular bronchitis Lymphocytic interstitial pneumonia
Bx: unclear respiratory distress syndrome in the non-neonate	

Clinical presentation

There are two main clinical scenarios of chILD: 1) an often dramatic and progressive respiratory failure in the perinatal period, or 2) a slowly progressive, but relentless dyspnoea either at rest or with exercise intolerance in older children. The term "chILD syndrome" has been established to identify a phenotype that requires further diagnostic evaluation. It is defined by the presence of at least three of the following four criteria: 1) respiratory symptoms (cough, rapid and/or difficulty breathing, exercise intolerance), 2) respiratory signs (resting tachypnoea, adventitious sounds, retractions, digital clubbing, failure to thrive, respiratory failure), 3) hypoxaemia and 4) diffuse abnormalities on chest radiography or high-resolution computed tomography (HRCT) [9]. Tachypnoea is the most common symptom (75–93%), followed by failure to thrive, cough, abnormal breathing sounds and hypoxaemia [2]. Before investigating for a specific chILD diagnosis, more common causes of childhood ILD have to be excluded. These include cystic fibrosis, acquired or congenital immunodeficiency, congenital heart disease, bronchopulmonary dysplasia, pulmonary infection, primary ciliary dyskinesia and recurrent aspiration [2, 9].

Diagnostic evaluation

The clinical evaluation includes a thorough medical history, including family, prenatal history and neonatal clinical course, analysis of oxygen saturation at rest and exercise testing, imaging (chest radiography and HRCT scan), blood studies focusing on immune function, autoantibodies, an echocardiogram, and lung function testing (for children aged >4–5 years) [2, 9, 12]. Helpful clinical protocols can be obtained at the chILD-EU website (www.childeu.net). Echocardiography serves to exclude structural cardiovascular disease and pulmonary hypertension (PH), which may be seen in up to 7% of children suspected of

suffering from ILD [9]. Although chest radiography rarely provides a specific chILD diagnosis, it may identify chILD mimics (especially infection), and helps to define the extent and pattern of structural lung alterations [15]. HRCT scanning is much more likely to accurately identify chILD; however, it needs to be done in specialised radiology departments to avoid poor imaging results. A strong correlation with histology is reported and thus HRCT has become the reference standard modality for radiological investigation [16]. The imaging results determine the further diagnostic work-up by allowing differentiation between 1) chILD present, reliably classifiable, 2) chILD present, but not reliably classifiable, and 3) chILD unlikely [17]. If imaging cannot render a definite and specific diagnosis, genetic testing and/or bronchoscopy with bronchoalveolar lavage (BAL) and lung biopsy may become necessary [10, 18]. The potential contribution of the different diagnostic modalities is outlined in the following sections, while a basic diagnostic algorithm employing these modalities is depicted in figure 1.

Genetic diagnosis

Molecular pathology has substantially contributed to the evolving classification of chILD; an association of genetic alterations with specific chILD entities has been reported in up to 20% of cases [19–22]. In a recent analysis of the chILD-EU register, 46% of the cases had genetic testing, with 13% of these allowing a final genetic diagnosis [23]. Genetic testing finds specific genetic alterations in up to 90% of cases with alveolocapillary dysplasia with misalignment of pulmonary veins (ACD/MPV), and 65% of cases with acinar dysplasia and congenital alveolar dysplasia (CAD), but may miss rare surfactant mutations depending on the molecular testing platform employed [19]. Nevertheless, early genetic testing to clarify the cause of respiratory dysfunction of the newborn may abrogate the necessity of performing video-assisted thoracoscopic surgery (VATS) biopsy; this is especially true for surfactant mutations and ACD/MPV [24, 25]. Thus, the indication for genetic testing should be made as early as possible in the

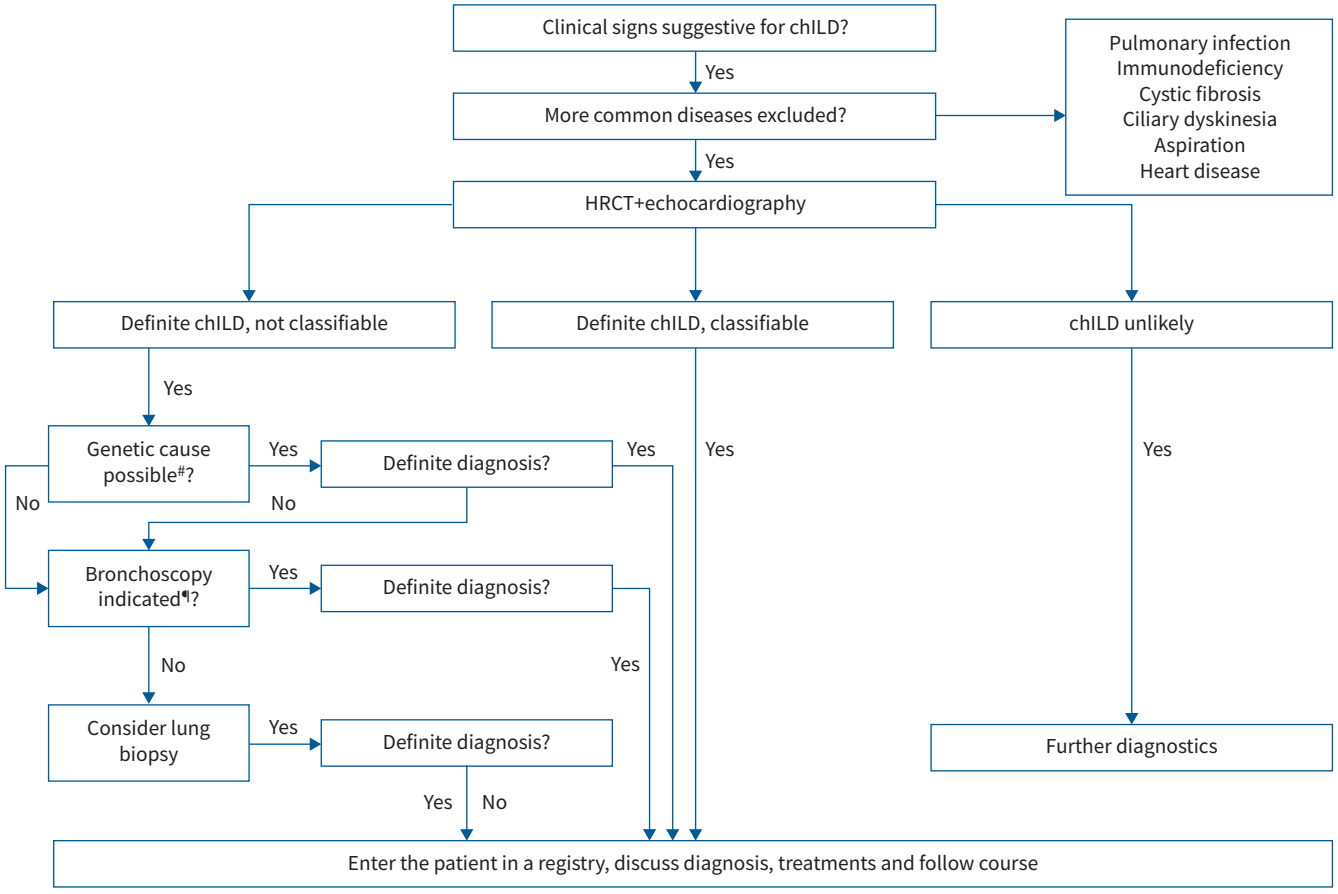

FIGURE 1 Diagnostic algorithm for the diagnosis of children's interstitial lung disease (chILD). A decision tree regarding the use of diagnostic procedures such as high-resolution computed tomography (HRCT), echocardiography, lavage and lung biopsy is shown. #: see flowchart in figure 2; ¶: to exclude infection, or to diagnose pulmonary alveolar proteinosis, diffuse alveolar haemorrhage storage diseases or hypersensitivity pneumonitis.

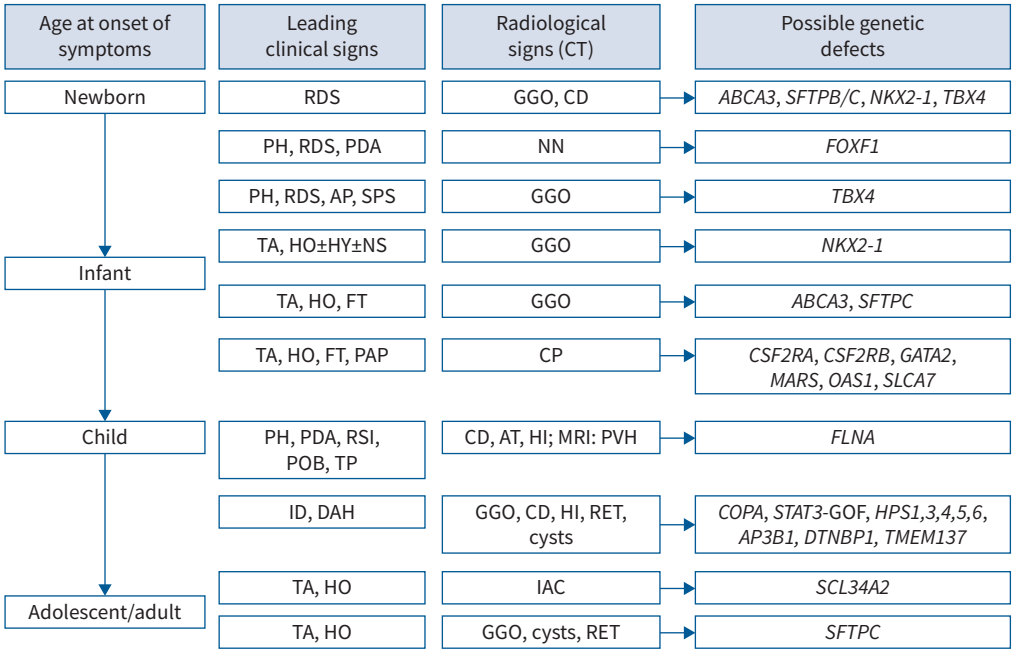

FIGURE 2 Diagnostic algorithm for specific genetic testing in children's interstitial lung disease (chILD). A decision tree depicting the association of clinical symptoms and radiological findings with specific genetic alterations is shown. RDS: respiratory distress syndrome; PH: pulmonary hypertension; PDA: persistent ductus arteriosus; AP: absent patella; SPS: small patella syndrome; TA: tachypnoea; HO: hypoxaemia; HY: hypothyroidism; NS: neurological signs; FT: failure to thrive; PAP: pulmonary alveolar proteinosis; RSI: recurrent severe infections; POB: pulmonary obstruction; TP: thrombocytopenia; ID: immune dysregulation; DAH: diffuse alveolar haemorrhage; CT: computed tomography; GGO: ground-glass opacification; NN: nearly normal; CP: crazy paving; CD: consolidations; AT: air trapping; HI: hyperinflation; MRI: magnetic resonance imaging; PVH: periventricular heterotopia; RET: reticulation; IAC: intra-alveolar calcification; GOF: gain of function.

diagnostic work-up of chILD in the neonate. A diagnostic approach incorporating clinical and radiological signs as well as the most frequent genetic alterations is given in figure 2.

Technically, next-generation sequencing (NGS)-based panels of disease-associated genes or exome/genome sequencing with respective filters are used. Sanger sequencing of single genes may be performed for confirmation or targeted diagnostics, if clinical symptoms or family history strongly support a distinct diagnosis (table 2). Account must be taken that in comparison to NGS, Sanger sequencing of single genes is a costly, inferior and rather outdated approach, and nowadays is primarily used if NGS sequencing is not available or waiting for the results would take too long. The vast majority of reported pathogenic variants in genes associated with chILD entities are single nucleotide variants or insertions/deletions (indels) of a few base pairs. However, in a few reported cases larger structural variants resulting in a (partial or complete) deletion or duplication of genes have been identified, especially in *TBX4* (T-box transcription factor 4) and *FGF10* (fibroblast growth factor 10) [26]. These genetic alterations may be undetectable with conventional short-read sequencing techniques. Thus, complimentary methods such as multiplex ligation-dependent probe amplification or array comparative genomic hybridisation may be informative in cases with regular sequencing results. The most common hereditary chILD entities and their associated genes are summarised in table 2.

Bronchoalveolar lavage

The diagnostic value of BAL in chILD has been addressed in a multitude of studies with inconsistent correlation of the findings with specific chILD entities [27]. Its primary usefulness is to exclude active infections and to provide samples for microbiological studies [9, 10]. For morphological analysis, conventional stains including haematoxylin/eosin, periodic acid–Schiff (PAS), iron and Sudan should be performed. Regarding the differential diagnosis of chILD, the value of BAL is hampered by the fact that cytological preparations primarily sample the proximal and distal airways and the alveoli, and changes of

TABLE 2 Children's interstitial lung disease (chILD) and specific genetic alterations (modified after [19–26])

Affected gene	Disease entity	Inheritance mode	Symptoms	Histology
ABCA3	Surfactant protein abnormality	Autosomal recessive	Respiratory symptoms from birth or gradually developing in later childhood	PAP, DIP, NSIP, CPI
COPA	COPA syndrome	Autosomal dominant	Immunodysregulation affecting lung, joints, kidney, *etc.*	Pneumonitis, capillaritis, alveolar haemorrhage
CSF2RA	PAP	X-associated	Dyspnoea and cough in early childhood	PAP
CSF2RB	PAP	X-associated	Dyspnoea and cough in early childhood	PAP
FLNA	Filamin A syndrome	X recessive	Dyspnoea in early childhood, periventricular heterotopia	Alveolar simplification
FOXF1	ACD/MPV	Autosomal dominant	Severe respiratory distress and pulmonary hypertension from birth	ACD/MPV
GATA2	PAP	Autosomal dominant	Opportunistic infections	PAP
MARS	Interstitial lung and liver disease	Autosomal recessive	Dyspnoea and cough in early childhood, lactate acidosis, liver cirrhosis, growth retardation	PAP
NKX2-1	Brain–lung–thyroid syndrome	Autosomal dominant	Dyspnoea from birth, recurrent infections	DIP, CPI, PAP, alveolar simplification
NSMCE3	Chromosomal breakage syndrome	Autosomal recessive	Growth retardation, immune deficiency, opportunistic infections	Infections (*e.g.* cytomegalovirus)
OAS1	Infantile onset PAP	Autosomal dominant	Virus-associated pneumonia, splenomegaly	PAP
SFTPB	Surfactant protein abnormality	Autosomal recessive	Severe dyspnoea from birth, rarely developing later	PAP, CPI
SFTPC	Surfactant protein abnormality	Autosomal dominant	Severe dyspnoea from birth, occasionally developing later	CPI, DIP, NSIP
SLCA7	Lysinuric protein intolerance	Autosomal recessive	Small stature, hepatosplenomegaly, infections, later dyspnoea	PAP, infections, rarely fibrosis, if present dismal prognosis
TBX4	Acinar dysplasia	Autosomal dominant	Severe dyspnoea from birth, pulmonary hypertension, subluxation of patella	Acinar dysplasia, NSIP, PIG
TMEM173	STING-associated vasculopathy	Autosomal dominant	Systemic inflammation with skin lesions, vasculopathy, dyspnoea	CPI, follicular bronchitis
SCL34A2	Familial pulmonary alveolar microlithiasis	Autosomal recessive	Dyspnoea and cough in childhood	Microlithiasis

PAP: pulmonary alveolar proteinosis; DIP: desquamative interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; CPI: chronic pneumonitis of infancy; ACD/MPV: alveolocapillary dysplasia with misalignment of pulmonary veins; PIG: pulmonary interstitial glycogenosis.

the cellular composition of the lung interstitium are not well represented [28]. Despite this limitation, BAL may give diagnostic clues in cases of alveolar haemorrhage [29], pulmonary alveolar proteinosis (PAP) [30, 31], sarcoidosis [32], Langerhans cell histiocytosis [33] or aspiration [34, 35]. Therefore, although the diagnostic yield of BAL may be limited, if a bronchoscopy is performed, it should be included in the diagnostic work-up.

The usefulness of the analysis of solutes in BAL is even more limited; the determination of the surfactant proteins SP-B (*SFTPB*) and SP-C (*SFTPC*) can, however, be of some value. The absence of SP-B is a strong indicator of *SFTPB* deficiency, and a low or absent level of SP-C suggests disturbance of type II pneumocyte surfactant metabolism and further genetic analysis is recommended [36]. The analysis of lavage lipid profiles is of interest for the development of disease biomarkers [37, 38].

Lung biopsy

Due to changes in technology, *e.g.* cryobiopsy and advances in genetic testing, there is an ongoing discussion on the use and timing of lung biopsy in chILD. A biopsy usually represents the final step in the diagnostic work-up (figure 1). In cases of rapidly progressive neonatal chILD with a suspected genetic cause or syndromic association, the biopsy may potentially render a diagnosis faster than the genetic analysis.

In contrast to adult patients, transbronchial biopsies are usually not performed as the small biopsy size limits the diagnostic yield. Nevertheless, there is consensus that surgical lung biopsies can achieve a specific diagnosis and guide further clinical management and therapy [9, 39]. Close communication

between the paediatrician, radiologist, surgeon and pathologist is mandatory in order to maximise diagnostic yield.

VATS has become the method of choice due to fewer post-interventional complications, a shorter recovery period and less pain for patients compared with conventional open lung biopsy [40]. Ideally, the biopsy should be taken from two different lobes of the lung, excluding the tip of the right middle lobe or the lingula, and measure at least 1 cm edge length to include bronchioles and muscular arteries [41].

Detailed instructions for the handling of the surgical specimen are available [41, 42]. A general scheme of the specimen handling should include samples for microbiology cultures, snap-frozen tissue for immunofluorescence and genetic studies, glutaraldehyde fixation for electron microscopy, and formalin fixation for conventional microscopy [41, 42]. Gentle distension of the specimen by inflation-fixation may provide a better preservation of the lung architecture. Step-by-step instructions for specimen handling are available on the chILD-EU website (www.childeu.net). Histopathological work-up should always include stains for matrix and fibres (trichrome staining) as well as a PAS stain. Other special stains for organisms and structural components should be performed as seen appropriate and not routinely ordered on all biopsies in order to save valuable tissue. As the chILD classification is aetiology based and histological diagnoses mainly rely on pattern recognition, the classification does not immediately provide a helpful diagnostic framework for pathologists. Table 3 summarises the most frequent histomorphological patterns and their associated clinical disease entities.

Diffuse developmental disorders of lung parenchyma (A1)

Diffuse disorders of lung development are characterised by morphological patterns recapitulating intra-uterine developmental stages. Affected children are usually symptomatic immediately after birth or within the first weeks of life. Physiologically, after an early embryonic stage with the formation of outpouchings of the foregut (weeks 4–7), the first 20 generations of airways are formed in the pseudo-glandular stage (weeks 5–17) followed by a canalicular phase (weeks 16–26) with lengthening and

TABLE 3 Morphological patterns of children's interstitial lung disease (chILD) and differential diagnosis

Pattern	Features	Possible diagnoses
Normal findings	Inconspicuous architecture of septa, interstitium and vessels	Neuroendocrine hyperplasia of infancy, regular lung
Alveolar maturation arrest	Pattern of canalicular or saccular lung development	Acinar dysplasia, congenital alveolar dysplasia
Alveolar simplification	Reduced number of enlarged alveoli without increase in cellularity	Chronic neonatal lung disease, chromosomal abnormalities, pulmonary hypoplasia
Interstitial widening with increased cellularity without inflammation	Ovoid PAS-positive cells (pulmonary interstitial glycogenosis)	Chronic neonatal lung disease, pulmonary interstitial glycogenosis, pulmonary hypertension, growth abnormalities
	Septal capillaries with central location, ectatic peribronchial veins	Alveolocapillary dysplasia with misalignment of pulmonary veins
Interstitial widening with inflammation	Type 2 cell hyperplasia, interstitial oedema, focal lymphoid infiltrates (chronic pneumonitis of infancy)	Surfactant dysfunction, virus infection, immunodeficiency
	Interstitial widening with dense lymphocytic inflammatory infiltrate and fibrosis (lymphocytic interstitial pneumonia)	Autoimmune disease, immunodeficiency
	Interstitial widening with discrete inflammatory infiltrate and fibrosis (nonspecific interstitial pneumonia)	Surfactant dysfunction in older children, autoimmune disease, hypersensitivity pneumonitis
Intra-alveolar filling	Inconspicuous septal architecture with PAS-D-positive intra-alveolar exudate	Surfactant dysfunction or nongenetic causes, immune dysfunction
	Dense intra-alveolar accumulation of macrophages (desquamative interstitial pneumonia)	Surfactant dysfunction, drug reaction, toxic inhalation
Airway obliteration	Fibrous remodelling of distal airways (obliterative bronchiolitis)	Post-infectious, chronic lung allograft dysfunction, graft *versus* host disease
Nodular lymphocytic infiltrates	Follicular bronchitis and bronchiolitis	Autoimmune disease, common variable immune deficiency
Granulomas	Variably distributed granulomas with/without necrosis	Infection, sarcoidosis, hypersensitivity pneumonitis, vasculitis, immune deficiency

PAS: periodic acid–Schiff.

widening of alveolar ducts [17, 43, 44]. By week 24 the terminal airways grow and widen to form alveoli in the saccular stage, which gradually blends into a phase of progressive alveolarisation continuing into early adulthood [17, 44]. The basal predominance of lung abnormalities in chILD may be explained by the propagation of all maturation processes from proximal to distal lung structures [44].

Acinar dysplasia is a very rare manifestation of a pulmonary maturation arrest resembling the pseudo-glandular or early canalicular phase. Most affected infants are female, born at term and cannot be oxygenated sufficiently even by mechanical ventilation, usually leading to death within hours after birth. A definite diagnosis usually requires an autopsy [1]. Both lungs appear small, and reveal only bronchi and occasionally bronchioles embedded in a loose mesenchyme completely lacking acini or alveoli [1, 45, 46]. Genetic alterations including *TBX4*, *FGF10* or *FGFR2* (fibroblast growth factor receptor 2) have recently been documented in up to 65% of affected infants with acinar dysplasia [26].

CAD is also a very rare maturation defect characterised by a morphological pattern resembling the late canalicular or early saccular phase. Affected infants are by definition born at term and require mechanical ventilation or extracorporeal membrane oxygenation immediately post-partum. The lungs are enlarged and heavy; histologically, a diffuse simplification of the alveolar architecture with broadened septa, a reduced number of alveolar capillaries and a predominance of type 2 pneumocytes are characteristic [1, 17, 45]. Using morphology alone, CAD cannot be reliably distinguished from nonspecific immaturity of the lung in pre-term infants, therefore CAD can only be diagnosed in term infants.

ACD/MPV is a rare cause of severe respiratory dysfunction. Most infants are born at term and are initially clinically unremarkable. Progressive respiratory dysfunction and therapy refractory, severe PH with right heart failure ensues in the first hours, days or even weeks and in some cases post-partum, which inevitably leads to death [1]. In contrast to other severe chILD forms manifesting within the first days or weeks after birth such as surfactant dysfunction disorders, acinar dysplasia or CAD, chest radiographs or CT scans often show only mild abnormalities. Moreover, compliance of the lung seems better with mostly adequate tidal volumes when children are ventilated. Congenital malformations of the heart, kidney or gastrointestinal tract are present in >80% of the affected children [47]. A causative *FOXF1* (forkhead box F1) mutation can be demonstrated in 40–90% of infants with ACD/MPV [47]. The only therapeutic option is lung transplantation. An early diagnosis by biopsy and/or genetic analysis is necessary to avoid doomed therapeutic interventions. Of note are recently observed cases with specific mutations without vascular misalignment revealing a more favourable course, challenging the paradigm of ACD/MPV being fatal [47]. Therefore, lung biopsy should be considered even if a *FOXF1* mutation is confirmed, especially when symptoms are less severe than expected in typical cases of ACD/MPV.

The histology in ACD/MPV shows four major morphological features: 1) reduced number of septal capillaries with central intraseptal location, 2) distended veins adjacent to pulmonary arteries in the peribronchial stroma, 3) media hyperplasia of small pulmonary arteries, and 4) ectasia of peribronchial and septal lymphatics (figure 3) [1, 17, 47]. By virtue of the small biopsy size and possible focal manifestation, sometimes not all of these features can be conclusively identified in a single biopsy. Initially, the pathognomonic peribronchial veins have been regarded as developmentally misplaced vessels. Recently it has been conclusively shown, that triggered by a disturbance of capillary development, embryonic bronchopulmonary anastomoses unfold and become massively ectatic, and thus the term venous misalignment is a misnomer [48, 49].

Diffuse growth abnormalities of lung parenchyma (A2)

All entities defined by alveolar growth abnormalities share incomplete or insufficient alveolarisation of prenatal or postnatal origin [50]. Most entities of this category manifest within the first year of life; the most frequent but rather nonspecific morphological pattern is alveolar simplification [7, 9]. The most common cause is pre-term delivery; in this clinical context the term chronic neonatal lung disease (CNLD) has been established for children requiring oxygen for >28 days after birth (figure 4) [51]. Comparable forms of alveolar simplification are the sequelae of pulmonary hypoplasia, either as a primary form associated with genetic abnormalities (*NKX2-1* (NK2 homeobox 1; alias *TTF1* (thyroid transcription factor 1)) deficiency, *FLNA* (filamin A) mutation, trisomy 21) (figure 5 and supplementary figure S1), or as a secondary form with impaired lung unfolding triggered by diaphragmatic hernia, oligohydramnios, thoracic skeletal dysplasia or neuromuscular disease (supplementary table S1 and figure 2) [7, 44, 45, 52].

Alveolar simplification usually becomes evident at low magnification and shows both enlargement as well as rounding of alveolar spaces with a reduced number of septa. It has to be differentiated from air trapping and emphysema. The so-called radial alveolar count (RAC), which documents the number of alveolar

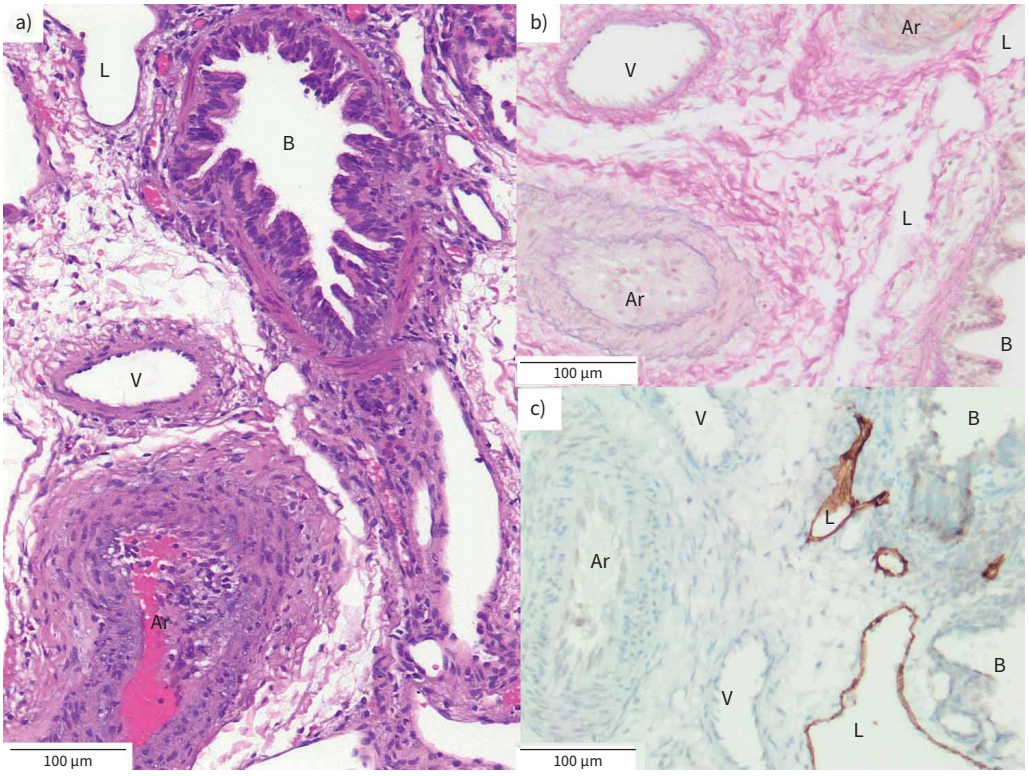

FIGURE 3 Lung biopsy of a mature neonate with alveolocapillary dysplasia with misalignment of pulmonary veins caused by *FOXF1* mutation. a) The pathological vasculature shows a membranous bronchus (B) accompanied by a triad of a peribronchial artery (Ar), a vein (V) and tortuous as well as ectatic lymphatics (L) (haematoxylin/eosin). b) Trichrome staining highlights the preserved elastica externa and interna of the pulmonary artery (Ar) and allows better separation of ectatic veins (V) with one-layered elastica. c) Immunohistochemical staining for podoplanin allows better separation of the lymphatics (L) from the arterial (Ar) and venous (V) vessels.

spaces between the centre of the most peripheral respiratory bronchiole and the nearest pleural surface or interlobular septum, has been used to objectify the degree of simplification. In small wedge biopsies its usefulness and necessity are debatable; the RAC value is ∼2 at 26 weeks, 5–6 by term and 9–10 by 10 years of age [53]. Additionally, a cellular interstitium with a pulmonary interstitial glycogenosis (PIG)-like pattern and hypertensive vascular changes may be observed. Morphologically, these findings are similar to a mild variant of bronchopulmonary dysplasia (BPD). Classic BPD has mostly been seen before the advent of artificial surfactant in premature infants treated with oxygen and ventilation [45]. It is morphologically defined by alternating areas of hyperinflation, collapse and alveolar simplification, squamous metaplasia, myogenous hyperplasia as well as periductal fibrosis, media hyperplasia of pulmonary arteries and focal obliterative bronchiolitis [10, 17, 18].

Specific entities of unknown aetiology (A3)

Pulmonary interstitial glycogenosis

PIG is the most common manifestation of a cellular, noninflammatory disorder of the lung interstitium. It may occur as an independent disease, but in most cases it is associated with other patterns of chILD such as alveolar simplification, ACD/MPV, PH or congenital lobular emphysema [45, 50, 51]. PIG as an independent chILD mostly manifests after a healthy interval with tachypnoea and oxygen desaturation within the first month of life [1]. Prognosis depends on the association with other forms of chILD; these associations are primarily responsible for the modification of the usually good prognosis with spontaneous resolution and good clinical response to corticosteroids.

PIG is morphologically characterised as a mostly diffuse, sometimes only focal widening of septa, with a predominance of ovoid or spindled cells with intracytoplasmic deposition of glycogen (figure 4) [1, 54]. Ultrastructurally these interstitial cells are organelle-poor lung-resident mesenchymal stem cells [55]. They

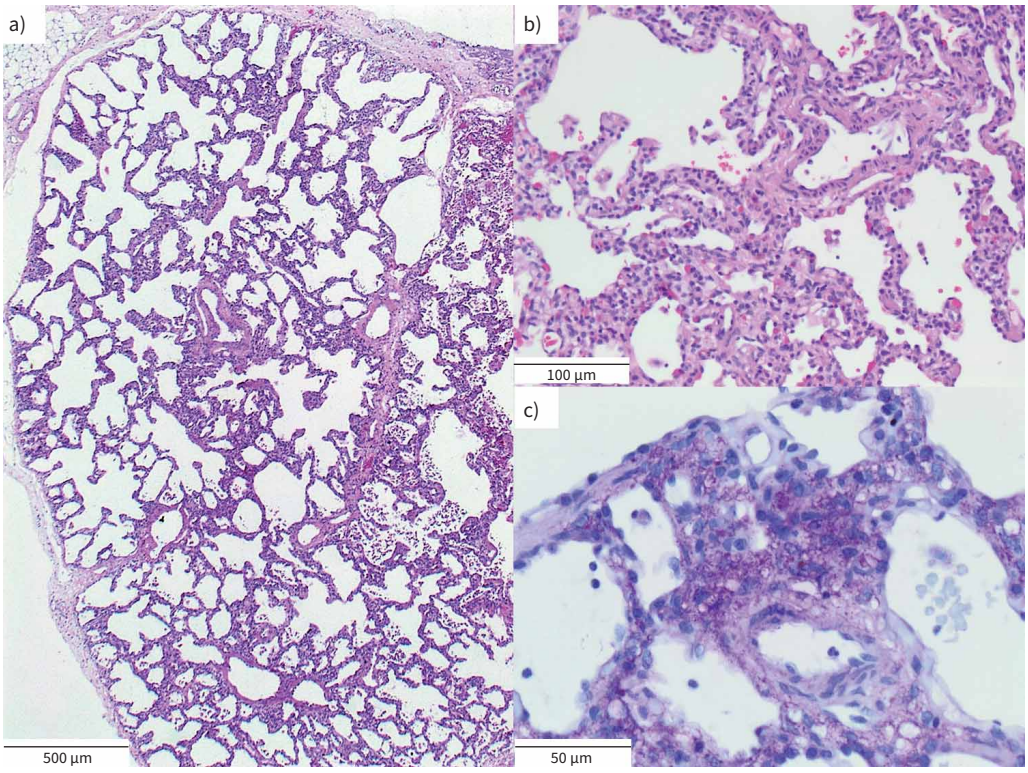

FIGURE 4 Lung biopsy of a 6-month-old male with chronic neonatal lung disease and pulmonary interstitial glycogenosis (PIG) caused by pre-term delivery and mechanical ventilation. a) At low power, architectural distortion with variably sized alveoli and subpleurally accentuated alveolar distension can be seen (haematoxylin/eosin). b) The alveolar septa appear widened and hypercellular without inflammatory infiltrates (haematoxylin/eosin). c) On periodic acid–Schiff (PAS) staining, the cellular interstitium reveals PAS-positive immature stromal cells corresponding to PIG.

have to be distinguished from fibroblasts as in BPD and inflammatory cells. The alveolar lining is inconspicuous, the alveolar spaces show no fluid or protein deposition, but an increased number of neuroendocrine cells may be seen [54]. Comparable findings have previously been termed histiocytoid pneumonia or infantile cellular interstitial pneumonitis. These terms are misnomers though, as a genuine inflammatory reaction is not part of PIG.

Persistent tachypnoea of infancy and neuroendocrine cell hyperplasia of infancy

The term persistent tachypnoea of infancy (PTI) was coined for children presenting with tachypnoea, retractions, hypoxaemia and failure to thrive [13, 17, 56, 57]. PTI is a relatively frequent but probably underdiagnosed form of chILD [17, 57]. It usually manifests within the first year of life and shows characteristic ground-glass opacities confined to the middle lobe, lingula and para-mediastinal areas in CT scans [58]. The severity of the clinical presentation is in contrast to the rather unremarkable morphology of the lung biopsy with usually only mild bronchitis and bronchiolitis [58]. This discrepancy of clinical symptoms and unremarkable morphology should always trigger immunohistochemical stains for neuroendocrine cells. Physiologically, neuroendocrine cells occur as single cells or in small clusters within the epithelium of bronchi or respiratory bronchioles [58, 59].

Neuroendocrine cell hyperplasia of infancy (NEHI) is defined by the presence of any number of neuroendocrine cells in at least 70% of all bronchi and a population of at least 10% in the epithelial lining of at least one bronchus [58, 59]. Bombesin is the most sensitive marker for neuroendocrine differentiation in this age group; in routine pathology more commonly used neuroendocrine markers such as chromogranin and synaptophysin give less reliable results [57–59]. The sensitivity of biopsies is hampered by the variable distribution of neuroendocrine cells in NEHI. Moreover, the specificity is confounded by the possible association of neuroendocrine hyperplasia with inflammatory and fibrosing lung diseases [59]. Increased neuroendocrine cells may also be interpreted as a form of lung immaturity [60]. Therefore, NEHI

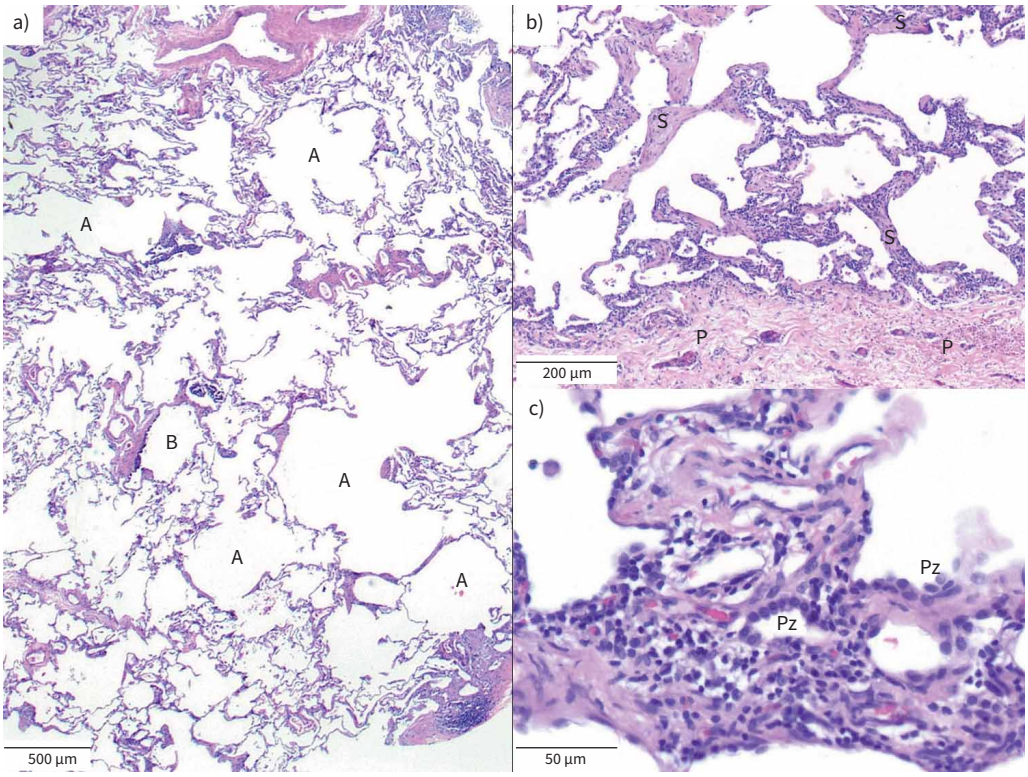

FIGURE 5 Lung biopsy from a 9-year-old male with alveolar simplification caused by *FLNA* mutation. a) Most striking is the massive and slightly variable enlargement of the alveolar spaces (A). Membranous bronchi (B) also appear dilated; the alveolar septa in the central parts of the lobuli show no widening or relevant inflammation (haematoxylin/eosin). b) The visceral pleura is thickened and fibrosed (P); the adjacent septa (S) are also broader and show a chronic inflammatory infiltrate (haematoxylin/eosin). c) The interstitium reveals a mixture of lymphocytes, plasma cells and occasional eosinophils. Focally, type 2 pneumocytes predominate the epithelial alveolar lining (Pz) (haematoxylin/eosin).

is a diagnosis of exclusion, and a thorough clinical history and HRCT scan are fundamental in making the correct diagnosis and are often sufficiently specific to avoid the need for surgical biopsy. Another diagnostic hint may be the lack of response to drugs such as corticosteroids, hydroxychloroquine or azithromycin typically used in the therapy of chILD. Nevertheless, in contrast to most other forms of chILD, the prognosis is excellent and most children become symptom-free once they enter school.

Mutations with surfactant dysfunction (A4)

Abnormalities of *SFTPB*, *SFTPC* and *ABCA3* (ATP-binding cassette family A member 3) are associated with up to 25% of clinically and morphologically variable, mostly severe forms of chILD [1, 17, 61–65]. Surfactant proteins, especially SP-B and SP-C, prevent alveolar collapse at the end of expiration by reducing surface tension at the air–water interface of the lung alveoli [17]. The process of synthesis, secretion and degradation of these proteins is highly regulated: surfactant gene transcription is dependent on thyroid transcription factor (*NKX2-1*) and subsequent intracellular storage and transport is regulated by the *ABCA3* protein, whereas surfactant processing and degradation by macrophages is under the influence of granulocyte–macrophage colony-stimulating factor (GM-CSF). Thus, alterations of any of these genes may contribute to surfactant dysfunction [17]. It should be noted that even identical genetic variants may show a divergent clinical course and morphology, suggesting the contribution of additional genetic and/or environmental factors [37, 62].

Both *SFTPB* and *ABCA3* mutations are inherited in an autosomal recessive pattern and most affected children develop respiratory failure in the neonatal period, with rapid disease progression and death at 3–6 months [37, 61–64]. In these infants, lung transplantation is the only treatment option. They show diffuse bi-pulmonary ground-glass opacities (“whiteout”) or a “crazy paving” pattern of mosaic ground-glass opacities in the imaging studies. More recently in cases of partial *SFTPB* expression, or a specific set of

ABCA3 mutations, milder courses have been observed [62, 66]. The inheritance pattern of *SFTPC* mutations is autosomal dominant with approximately half of the mutations *de novo*. They show a variable penetrance and severity, with manifestations ranging from severe respiratory distress in infants to idiopathic pulmonary fibrosis in older children and adults.

Morphological findings in patients with surfactant disorders consist of a variable combination of the following patterns: 1) diffuse hyperplasia of type 2 pneumocytes, 2) PAP consisting of a protein-rich PAS-D-positive intra-alveolar exudate (supplementary figure S2), 3) desquamative interstitial pneumonia (DIP) with accumulation of intra-alveolar xanthomatous macrophages (figure 6), 4) nonspecific interstitial pneumonia (NSIP) with interstitial widening, fibrosis and slight lymphocytic infiltrate, and 5) chronic pneumonitis of infancy (CPI) with interstitial lymphocytic infiltrates, a slightly fibrotic and widened septal interstitium, and type 2 pneumocyte hyperplasia [50, 61–63]. Ultrastructurally, *SFTPB* mutations show multivesicular and multilamellated lamellar bodies, whereas *ABCA3* mutations are associated with a reduced number of small lamellar bodies with pathological electron-dense bodies giving a “fried egg” appearance.

In the perinatal period, PAP in combination with type 2 cells, hyperplasia and mild interstitial inflammation is the most common finding. The presence of a PAS-D-positive exudate should raise the possibility of a surfactant disorder at any age. With increasing duration of the disorder, the exudate becomes more granular and permeated with cholesterol clefts and giant cells. NSIP and CPI patterns with distortion of the lung architecture and discrete fibrosis are more characteristic of milder forms of surfactant dysfunctions, usually seen in older children and more often associated with *SFTPC* or a specific subset of *ABCA3* mutations. There is only a loose association of specific genetic alterations with morphological patterns (see previous paragraph): 1) PAP is associated with *ABCA3* and *SFTPB* alterations, 2) CPI can be demonstrated primarily with *SFTPC* and *ABCA3* alterations (supplementary figure S3), and 3) a DIP pattern is seen often with *ABCA3* and *SFTPC* alterations (figure 6) [1, 43, 50, 61].

Pulmonary alveolar proteinosis

PAP is defined by an intra-alveolar accumulation of surfactant, triggered by a disturbance of surfactant homeostasis with increased protein expression, reduced protein degradation or both [25]. Morphologically, the intra-alveolar deposition of eosinophilic, rather cell-free finely granular material with cholesterol clefts is typical [67]. Additionally, hyperplasia of type 2 pneumocytes, xanthomatous macrophages and neutrophils may be found, often correlating with the duration of the disease. The interstitium is usually unremarkable; at most a slight inflammatory infiltrate may be seen. The diagnosis is often made already during BAL by noting the characteristic milky properties of the retrieved fluid; therefore, the result of the biopsy is only confirmatory. If PAP presents in the perinatal period, genetic alterations of *SFTPB*, *SFTPC*, *ABCA3* or *TTF1* predominate (supplementary figure S2) [25, 67]. In contrast, PAP manifesting in older children is caused by *CSF2RA* (GM-CSF receptor α), *CSF2RB* (GM-CSF receptor β) or *OAS1* (2′-5′-oligoadenylate synthetase 1) mutations, haematological neoplasia, metabolic disturbances (*e.g.* *SLC7A7* (solute carrier family 7 member 7; lysinuric protein intolerance)), infections (cytomegalovirus, respiratory syncytial virus), GM-CSF autoantibodies (as in most adult forms) and inhalation of inorganic dust [30, 31, 67]. In these forms of PAP the intra-alveolar exudate is more homogeneous and less granular; moreover, there will be less pneumocyte hyperplasia and alveolar remodelling. Whole-lung lavage may be used as a stabilising therapy [25, 67–69].

ILD related to systemic disease processes (B1)

Storage diseases

While in most cases the diagnosis of a storage disorder will already have been established, in some cases lung involvement may prove to be the first clinical manifestation [70]. The most common storage disorders are Niemann–Pick disease [71], Gaucher’s disease [72], glycogen storage disease, and mucopolysaccharidoses and mucolipidoses [73]. Lysosomal and glycogen storage disorders typically manifest within the lung as intra-alveolar and interstitial infiltrates of foamy or vacuolated macrophages. To start with, the finding of intra-alveolar macrophages is rather nonspecific and may be seen in aspiration, airway obstruction or surfactant dysfunction. However, macrophages demonstrable in the septal or pleural compartments are an important clue to the manifestation of a storage disease or histiocytic proliferation, as seen in Langerhans cell histiocytosis or Erdheim–Chester disease. Immunohistochemistry and electron microscopy may be helpful in the differential diagnosis.

Autoimmune/rheumatological disease

There is a wide spectrum of autoimmune/rheumatic diseases with lung involvement that may occur in mostly older children and adolescents. Pulmonary disease can be associated with rheumatic disease, but the incidence and presentation varies substantially between the disease entities and may be less frequent than

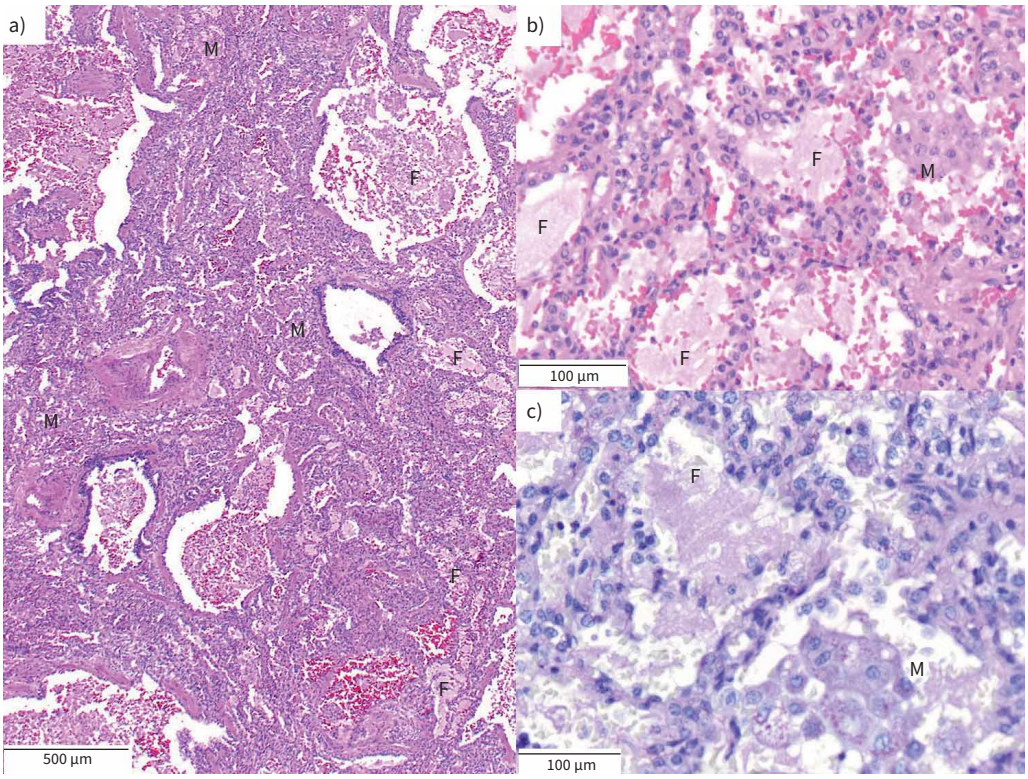

FIGURE 6 Lung explant from a 2-year-old male with chronic pneumonitis of infancy and desquamative interstitial pneumonia (DIP) caused by *SFTPC* mutation. a) At low power, a mixture of morphological patterns can be appreciated: widened and hypercellular septa encase alveoli with a DIP-like intra-alveolar accumulation of macrophages (M) and an alveolar proteinosis-like fluid deposition (F) (haematoxylin/eosin). b) The macrophages reveal a vacuolated cytoplasm; in the septal interstitium, immature stromal cells predominate with only sparse accompanying inflammatory cells. The epithelial lining shows type 2 cell hyperplasia (haematoxylin/eosin). c) On periodic acid–Schiff (PAS) staining, the cellular interstitium reveals sparse PAS-positive stromal cells; the alveoli again demonstrate a partial filling by macrophages (M) and exudate (F).

in adult disease [74]. The strongest association is with connective tissue disease followed by autoimmune processes triggered by inborn errors of immunity (IEI) (see later section on "ILD of the immunocompromised host (B3)"), vasculitis and sarcoidosis. Most of these entities have been extensively documented in the adult literature; supplementary table S2 summarises the most important diseases and their morphological manifestations [74–76]. Interstitial lung fibrosis as the most devastating pulmonary manifestation is mostly seen in systemic sclerosis [64]. The diagnostic challenge for clinicians and pathologists consists of bearing these rare causes of pulmonary dysfunctions in mind. Overlapping effects of treatment-related side-effects or infection may further complicate an adequate diagnosis [74].

ILD associated with exposure in the normal host (B2)

Hypersensitivity pneumonitis is the most frequent form of chILD in this group, mainly affecting children aged >2 years and adolescents [77]. Hypersensitivity pneumonitis is well described in the adult literature and the diagnostic criteria have been recently updated [78]. In children there are less documented and known triggers, with bird fancier's disease and humidifier lung disease as the most frequent forms, and chemical lung disease by inorganic antigens less frequent than in adults [10, 76, 77]. Symptoms of hypersensitivity pneumonitis in childhood may include dry cough, dyspnoea, asthenia and poor growth [76], but a correct diagnosis is often only rendered after prolonged exposure to high levels of inhaled antigens [77]. Diagnosis rests on a thorough clinical history, specific serum-precipitating IgG antibodies and disease regression after exposure removal. BAL and transbronchial biopsy may be helpful, especially if a characteristic mixed pattern of lymphocytic bronchiolitis, organising pneumonia, small and poorly formed granulomas with giant cells, and interstitial pneumonitis can be demonstrated (supplementary figure S4). Corticosteroid therapy resolves the symptoms promptly, but significantly alters the morphology by reducing the degree of mesenchymal proliferation and inflammation.

Acute respiratory viral illness is commonly diagnosed by clinical, serological and/or molecular pathological studies; therefore, there is usually a limited role for lung biopsy. In cases of viral infection, a nonspecific interstitial lymphocytic infiltrate with lymphocytic bronchitis and epithelial necrosis may be seen. Specific viral cytopathic effects may be seen with herpes simplex, adenovirus and cytomegalovirus infection, although typical nuclear alterations may be lacking if virostatic therapy has already been initiated [79].

Obliterative bronchiolitis in children is mostly triggered by a dysfunctional healing following viral (*e.g.* adenovirus) or bacterial (*e.g. Mycoplasma pneumoniae*) infections. However, it can also be caused by autoimmune processes, chronic aspiration or autoimmune disease and a sequela of lung or bone marrow transplantation [80–82]. It presents with obstructive lung dysfunction and mosaic perfusion in CT scans corresponding to air trapping and ventilation–perfusion mismatch [80, 81]. Obliterative bronchiolitis is morphologically defined by a progressive obliterative remodelling of mostly peripheral airways, commencing with subtle band-like subepithelial fibrosis and leading to complete luminal obstruction by dense fibrous plugs. The findings may be subtle, and close examination of pulmonary vasculature looking for unpaired arteries and secondary findings such as airspace distension, foamy macrophages and cholesterol clefts are helpful [80, 81]. As the distribution of obliterated airways may be inhomogeneous and primarily manifest in more distal airways, surgical lung biopsy of at least two lung areas is usually necessary to confirm the diagnosis of suspected obliterative bronchiolitis.

ILD of the immunocompromised host (B3)

Errors of immunity may be acquired (AEI) or inborn (IEI), with most acquired forms in childhood caused by chemotherapy, stem cell or solid-organ transplantation. Regarding IEI, more than 400 different entities have been described, with a growing number of associated monogenic germline mutations [83, 84]. More than half result in antibody deficiency, but the vast majority of patients with antibody deficiency lack defined genetic defects and carry the diagnosis of common variable immune deficiency (CVID) with low IgG, IgA and/or IgM levels [84, 85]. Due to the better understanding of the pathophysiology and the recognition that there is often a combination of immunodeficiency and auto-inflammation or immune dysregulation, the previous term “primary immunodeficiency” has been replaced by the term “IEI” [83]. Regardless of its specific cause in chILD triggered by errors of immunity, respiratory disease is a relevant cause of morbidity and mortality [86–88].

Both AEI as well as IEI may trigger airway remodelling. Remodelling of the mostly distal airways in the form of obliterative bronchiolitis may occur either post-infection or result from alloimmune reactions in stem cell or lung transplanted patients [80–82]. The histomorphology is the same; however, in all cases other than post-infection, obliterative bronchiolitis is often progressive. In these clinical settings, lung biopsy is part of the diagnostic work-up, in particular when searching for poorly identifiable infectious agents such as fungi. Chronic granulomatous lung disease is an example of an IEI triggered by a *CYBB* (cytochrome B-245 β chain) mutation presenting as multifocal granulomas containing mycotic pathogens (figure 7). Bronchiectases, on the other hand, mainly affect the more proximal airways and are mostly the sequelae of noncontrolled chronic infection [87]. Diagnosis rests on HRCT or magnetic resonance imaging since chest radiography has a sensitivity of <30% [87]. Biopsies are usually not part of the diagnostic work-up, but may show nonspecific ulceration and scarring.

Errors of immunity are often associated with different forms of chILD, possibly as a result of immune dysregulation, and not strictly and exclusively related to infectious complications [85–87]. In the most comprehensively studied group of CVID, ILD is observed in 10–60% of patients; the wide range is possibly a result of selection bias and a variable frequency of lung function testing and imaging [89–92]. In CVID patients, enteropathy and a variety of other immune-related disorders, such as coeliac disease, pernicious and haemolytic anaemia, thyroiditis, hepatitis, and arthritis, may accompany lung disorders [90]. Previously, granulomatous-lymphocytic interstitial lung disease (GLILD), consisting of a combination of noncaseating granulomas and lymphoid proliferations, has been introduced as a characteristic pattern in these patients [93], although detailed analyses have shown that GLILD is an umbrella term encompassing a broad spectrum of errors-of-immunity-associated lung pathology. Thus, it should be avoided and a concise description of the injury patterns is preferred [87, 90]. The following lung injury patterns may be seen in errors of immunity in variable combinations (supplementary figures S5 and S6): 1) noncaseating preferably intra-alveolar compact granulomas sometimes accompanied by plasma cells, 2) lymphoid hyperplasia consisting of peribronchial lymphoid infiltrates with or without germinal centres, diffuse forms of lymphoid hyperplasia, nodular lymphoid hyperplasia, lymphocytic interstitial pneumonia or NSIP patterns, 3) organising pneumonia pattern with and without accompanying granulomas, 4) bronchiectasis mostly associated with the presence of granulomas, and 5) rarely interstitial fibrosis [88, 90, 91, 94, 95]. In summary, there are no morphological features specific for errors of immunity, but finding any of the

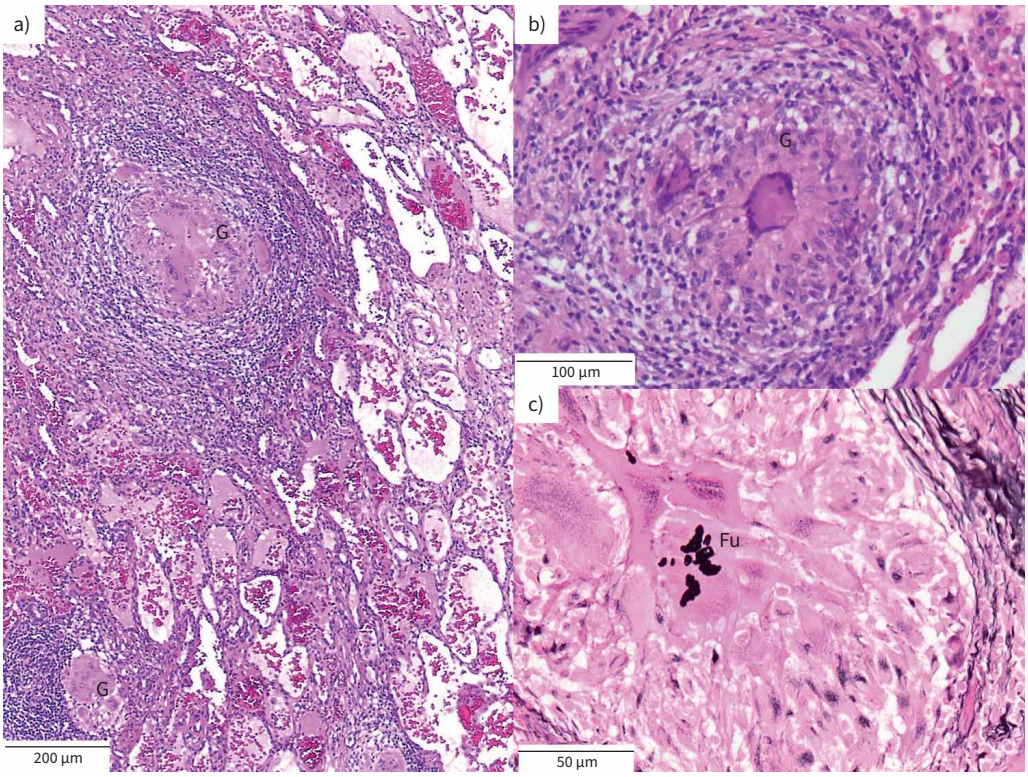

FIGURE 7 Lung biopsy of a 6-year-old male with chronic granulomatous disease caused by *CYBB* mutation. a) At low power, granulomas (G) of variable size with an accompanying inflammatory infiltrate are unevenly distributed in the parenchyma (haematoxylin/eosin). b) Multinuclear giant cells are surrounded by histiocytes and a wreath-like lymphocytic infiltrate (haematoxylin/eosin). c) Silver staining (Gomori) reveals fragmented fungal hyphae within the giant cells (Fu); *Aspergillus niger* could be demonstrated by molecular analysis.

aforementioned patterns, especially in combination, should instigate a thorough clinical history and Ig level testing of the patients.

ILD with structural vascular changes (B4)

Pulmonary vascular disease may mimic ILD clinically and by imaging. Pulmonary vascular remodelling represents, in the broadest sense, a common feature in ILD. It occurs mostly secondary to CNLD, lung hypoplasia and underlying congenital heart disease. Primary and familial forms of pulmonary arterial hypertension (PAH) are rare in infants, typically manifesting in older children or adolescents. Morphology, *e.g.* intimal sclerosis and/or medial hypertrophy of the pulmonary vasculature, does not correlate well with the presence and/or grade of PH. Therefore, histological assessment is rarely on the forefront of diagnostics in PAH, as first-line investigations include cardiac ultrasound, vascular catheterisation, HRCT and blood biomarkers [96].

Nevertheless, lung biopsies can be of substantial value to differentiate PAH from pulmonary veno-occlusive disease (PVOD), which show substantial overlap in haemodynamic findings [97]. Depending on the severity and temporal evolvement of PAH, histomorphological features range from medial and intimal hyperplasia, (sub)total fibrotic luminary occlusion, referred to as concentric lesions, dilatation lesions and formation of plexiform lesions (complex, glomeruloid-like vascular structures) to necrotising arteritis. All of these can be classified according to the (revised) Heath–Edwards or Rabinovich systems [98]. In contrast, PVOD is characterised by distinct fibrotic narrowing of pulmonary venules/veins, frequently accompanied by pulmonary capillary haemangiomatosis (PCH). PCH may resemble severe congestion, but is actually a marked abnormal proliferation of pulmonary capillaries characterised by a genuine multiplication of vascular channels in the alveolar wall, which may extend into the walls of arterioles and venules (supplementary figure S7). PCH was previously thought to occur only in conjunction with PVOD, but has recently been shown to also occur independently. As in P(A)H, pulmonary haemorrhage of varying extent with haemosiderin-laden macrophages is frequent in PVOD/

PCH (supplementary figure S7) [97]. Pulmonary venous hypertension may not only manifest in PVOD, but also occurs with pulmonary vein stenosis or left ventricular heart failure. Morphologically, it is characterised by muscularisation and/or arterialisation of the pulmonary veins mostly accompanied by severe pulmonary artery disease.

ALK1 (activin A receptor like type 1), *BMPR2* (bone morphogenetic protein receptor type 2), caveolin, *TBX4* (T-box transcription factor 4), *KCNK3* (potassium two pore domain channel subfamily k member 3) and *SMAD9* (SMAD family member 9) mutations have been shown to be involved in the genesis of (familial) PAH, accounting for about one-third of paediatric PAH cases. In PVOD, mutations of *EIF2AK4* (eukaryotic translation initiation factor 2α kinase 4) have been linked to disease genesis in sporadic and familial cases alike. More severe clinical symptoms at the time of diagnosis appear to be a common denominator in mutation carriers in PAH and PVOD, alike [96, 97, 99, 100].

Support by peer experts: the chILD-EU management platform

As the majority of rare diseases are diagnosed and treated in general paediatric hospitals and offices, a close connection of these sites to specialised national or international centres is warranted. The international management platform for chILD (chILD-EU) carries out independent multidisciplinary reviews of chILD cases. Documentation of follow-up data and a bio-archive support individual and group investigations in an open way [23]. Data analysis of the first 3 years (January 2014 to November 2016) showed that the initial diagnosis was not confirmed by peer review in 13% of the cases [23]. This platform generated a solid basis for the comprehensive care of paediatric ILDs and now offers an important base for novel molecular studies, investigations on the natural history, and opportunities for the development of novel pharmaceutical treatments and their monitoring in a centralised registry.

Provenance: Submitted article, peer reviewed.

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