Diagnostic workup of childhood interstitial lung disease

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Shareable abstract (@ERSpublications)
Childhood interstitial lung diseases are rare and severe diseases. A stepwise approach to an aetiological diagnosis includes specific investigations performed in expert centres. The term "undefined chILD" must be regularly reassessed. https://bit.ly/3YIWKvn

Abstract

Childhood interstitial lung diseases (chILDs) are rare and heterogeneous diseases with significant morbidity and mortality. An accurate and quick aetiological diagnosis may contribute to better management and personalised treatment. On behalf of the European Respiratory Society Clinical Research Collaboration for chILD (ERS CRC chILD-EU), this review summarises the roles of the general paediatrician, paediatric pulmonologists and expert centres in the complex diagnostic workup. Each patient's aetiological chILD diagnosis must be reached without prolonged delays in a stepwise approach from medical history, signs, symptoms, clinical tests and imaging, to advanced genetic analysis and specialised procedures including bronchoalveolar lavage and biopsy, if necessary. Finally, as medical progress is fast, the need to revisit a diagnosis of "undefined chILD" is stressed.

Introduction

Rare lung diseases in children comprise a variety of conditions that include cystic fibrosis, primary ciliary dyskinesia, congenital malformations of the lung, pulmonary hypertension, abnormal ventilatory drive and childhood interstitial lung diseases (chILDs). The latter is, by itself, a heterogeneous group of very rare lung diseases with an overall estimated prevalence of 1.6–46 per million depending on the few available reports [1–13]. Thus, they appear to be around 10 times rarer than in adults, covering different aetiologies with some of them being extremely severe [14]. Most general practitioners and paediatricians will face none or one of these patients in their whole career and even paediatric pulmonologists may manage only a few cases of chILD. Unspecific and often inconspicuous, clinical signs could also delay the diagnosis and worsen the prognosis for chILD [1, 5]. When an ILD in a child is suspected, further investigations should be performed by experienced radiologists, geneticists and pathologists. Despite an exhaustive workup, a proportion of 6–12% of chILD remains unexplained or undefined [5, 11, 15].

In the past years, most European countries have identified specialised clinicians in expert centres who developed a chILD network. The Clinical Research Collaboration for chILD-EU, supported by the European Respiratory Society (ERS CRC chILD-EU), focuses on improving the diagnosis and management of chILD. The present review provides an up-to-date overview of the stepwise diagnostic process of chILD, from the involvement of the general paediatrician to the minimal set of required tests performed in expert centres, and discusses the current definition of "undefined chILD".

chILD definition and classification

The diagnosis of chILD has been defined as the presence of at least three of the following criteria: 1) respiratory symptoms, 2) clinical signs of respiratory insufficiency, 3) hypoxaemia or low pulsed oxygen saturation and 4) diffuse parenchymal lung disease on chest radiography or thoracic computed tomography (CT) scan [16, 17].

Respiratory symptoms and clinical signs of chILD are non-specific. They include tachypnoea, dyspnoea on exertion (such as feeding in infants/neonates) or at rest, persistent dry cough, failure to thrive, retractions, crackles, digital clubbing, cyanosis and, less frequently, chest wall deformity (pectus excavatum and others) [17]. Other clinical signs that can point to a chILD aetiology are haemoptysis, presence of pulmonary hypertension, signs of peripheral hypothyroidism, recurrent fever, skin lesions, joint pain and/or neurological issues such as hypotonia, developmental delay, chorea and sensorial defects [18, 19]. In neonates, an unexplained respiratory distress, especially in a term baby, should also raise suspicion of chILD [20, 21].

Based on this definition, the general paediatrician facing a patient with persistent respiratory symptoms consistent with chILD should actively pursue this diagnosis to identify such rare cases from the bulk of recurrent respiratory tract infections and other more frequently occurring entities. Detailed history, description of findings at examination and a chest radiograph should be performed before referring the patient to a paediatric pulmonologist or a paediatric pulmonary department. These specialists will exclude differential diagnoses, confirm the diagnosis of chILD and push forward the investigations to try to define the chILD aetiology.

The 2004 report of the ERS Task force on chronic ILD in immunocompetent children presented the first classification system for children that was closely linked to the classification system in adults [22]. In 2007, pathologists, together with clinicians, proposed a classification system based on the histology of lung tissue for children <2 years of age [15]. This system was later extended to all paediatric age groups. Altogether, the main identified groups of chILD are 1) ILD related to primary parenchymal disorder (mostly group A of the chILD-EU classification [23]), 2) ILD specific to infancy, 3) ILD related to systemic disease processes and 4) ILD related to exposure/environmental insults [23–28]. Among these categories, the most frequent diagnoses are neuroendocrine cell hyperplasia of infancy (NEHI) (also called persistent tachypnoea of infancy (PTI) in the absence of histology), inherited surfactant disorders, diffuse alveolar haemorrhage, pulmonary alveolar proteinosis, sarcoidosis, autoinflammatory diseases and connective tissue diseases. These disorders are highly heterogeneous, but sometimes present similarly and as a consequence may be difficult to diagnose. More than 50 conditions can be associated with a chILD and deciphering which exact aetiology is affecting the patient can be long and unsuccessful, emphasising the importance of a systematic diagnostic workup.

Diagnostic workup for chILD

Over the past decade, USA and European Union work groups have proposed some diagnostic approaches [16, 17]. The first was by KURLAND et al. [16] in 2013, in the framework of the American Thoracic Society, and was based on a careful family screening for ILD, followed by the elimination of other diagnoses before proceeding to more specific chILD investigations such as CT scan, genetic tests and lung biopsy. At that time, the number of involved genes was limited to surfactant-related genes (SFTPB, SFTPC, ABCA3 and NKX2-1), pulmonary alveolar proteinosis genes (CSF2RA and CSF2RB) and FOXF1 for diffuse abnormalities of lung development. This first publication was of major help, and allowed improved diagnosis and classification of ILD in children. Two years later, BUSH et al. [17] on behalf of the chILD-EU working group proposed another flowchart for the diagnosis of chILD, primarily based on CT scan and placing blood tests, especially genetic testing, before more invasive tests such as bronchoalveolar lavage and lung biopsy. The genetic evolution reflected the expansion and the wider availability of new molecular techniques allowing the study of a panel of genes (next-generation sequencing (NGS) and whole-exome sequencing (WES)) instead of one by one (Sanger sequencing). This led to the discovery of new genetic entities in chILD, such as MARS mutations, other cytosolic aminoacyl-tRNA synthetase (ARS) mutations or OAS1 in pulmonary alveolar proteinosis [29–33], COPA and STING1 mutations for

ILD related to autoinflammatory disorders [34–36], and many other even rarer diseases related to mutations in *FLNA, TBX4, NHLRC2* or *ZNFX1* [25, 37–41].

The multiplication of new aetiologies has raised the question of prioritisation of the diagnostic approach, from the clinical approach to the choice and timing of genetic tests, but also the place of lung biopsy (figure 1a) and finally, when are we allowed to label a patient as "undefined chILD"?

Medical history, family screening and careful clinical examination

This remains the first and major step of chILD workup as valuable information can be retrieved from the patient and their family history.

Establishing a genealogical tree, also called a pedigree chart, is mandatory in all chILD. It is estimated that up to 20–30% of chILDs are due to monogenic diseases, some of them being associated with extrapulmonary involvement. Thus, collecting information on relatives and siblings can be highly useful: oxygen therapy, lung transplantation, neonatal respiratory distress or unexplained death, neurological issues such as hypotonia, developmental delay, chorea (*NKX2-1*), cerebral aneurysms (*FARSA* and *FARSB*), sensorial defects (ARS), peripheral hypothyroidism (*NKX2-1*), autoimmune diseases or general symptoms such as fever, skin lesions, joint pains (autoinflammatory disorders, connective tissue diseases), age and cause of death of older generation family members may be of interest. Consanguinity is also very important to be aware of, as it could increase the risk of rare recessive homozygous disease (*ABCA3*, *MARS*/other ARS genes and *SFTPB*).

The age at onset of the ILD is crucial information. Even though it is now well documented that almost all chILD can occur at any age, some diagnoses are much more frequent in newborns, infants or older children, as shown in figure 1b [42–44].

The habits and living conditions of the patient can orientate one to chILD related to lung toxicity (*e.g.* drugs, medications and radiation) or hypersensitivity pneumonitis and other exposure-related diseases (*e.g.* birds, hay, mould and air conditioners).

CT scan: the cornerstone of chILD diagnosis

As far as the patient's medical status makes it possible, and when the diagnosis of chILD is suspected, a high-resolution CT (HRCT) scan is the first-line investigation to be performed [45, 46]. The HRCT scan will allow to confirm ILD and to identify the ILD pattern. As the technique and the interpretation of the HRCT scan are crucial, it should be performed in expert centres for paediatric imaging using protocols for optimisation of image quality while keeping individual radiation exposure to the lowest level (especially in neonates and infants) [47–49]. The use of intravenous contrast is indicated if lymphadenopathies, gross structural abnormalities, or associated cardiac or vessel abnormalities need to be differentiated. The lung parenchyma analysis will search for elementary lesions of ILD such as ground-glass anomalies, consolidations, thickening of the bronchovascular interstitium, thickening of the interlobular septa, visualisation of intralobular lines, cystic lesions and micronodules or nodules. Their association, distribution, extent as well as the presence of signs of fibrosis will be sought [50, 51].

In the majority of cases, the picture remains non-specific, *i.e.* it is helpful for general ILD diagnosis but unable to identify a specific aetiology. The CT pattern observed varies depending on the age of the child. Infants most often present with diffuse ground-glass anomalies associated or not with other abnormalities/findings. Older children may have more cystic, nodular or even fibrosing abnormalities. However, in specific cases, the aetiological diagnosis may be guided by typical lesions, such as images of "crazy paving" favouring alveolar proteinosis or other patterns detailed in table 1.

Gas exchange and pulmonary function tests

Oxygen saturation at rest, during sleep and with exercise, the absence or presence of clinical signs, and pulmonary hypertension are used in the Fan severity score for chILD (rated 1 (low severity) to 5 (high severity)) [52]. Blood gas may be of interest to determine impairment of gas exchange. The 6-min walk test is particularly interesting in chILD because of its high sensitivity and ease of use from the age of 4–5 years [53].

The first pulmonary function tests (PFTs) should be performed as soon as possible after chILD diagnosis, if the child's condition allows it and depending on their age [54–60]. Although testing seldom provides an aetiology to the ILD, it allows to objectively assess the functional consequences of the pathology.
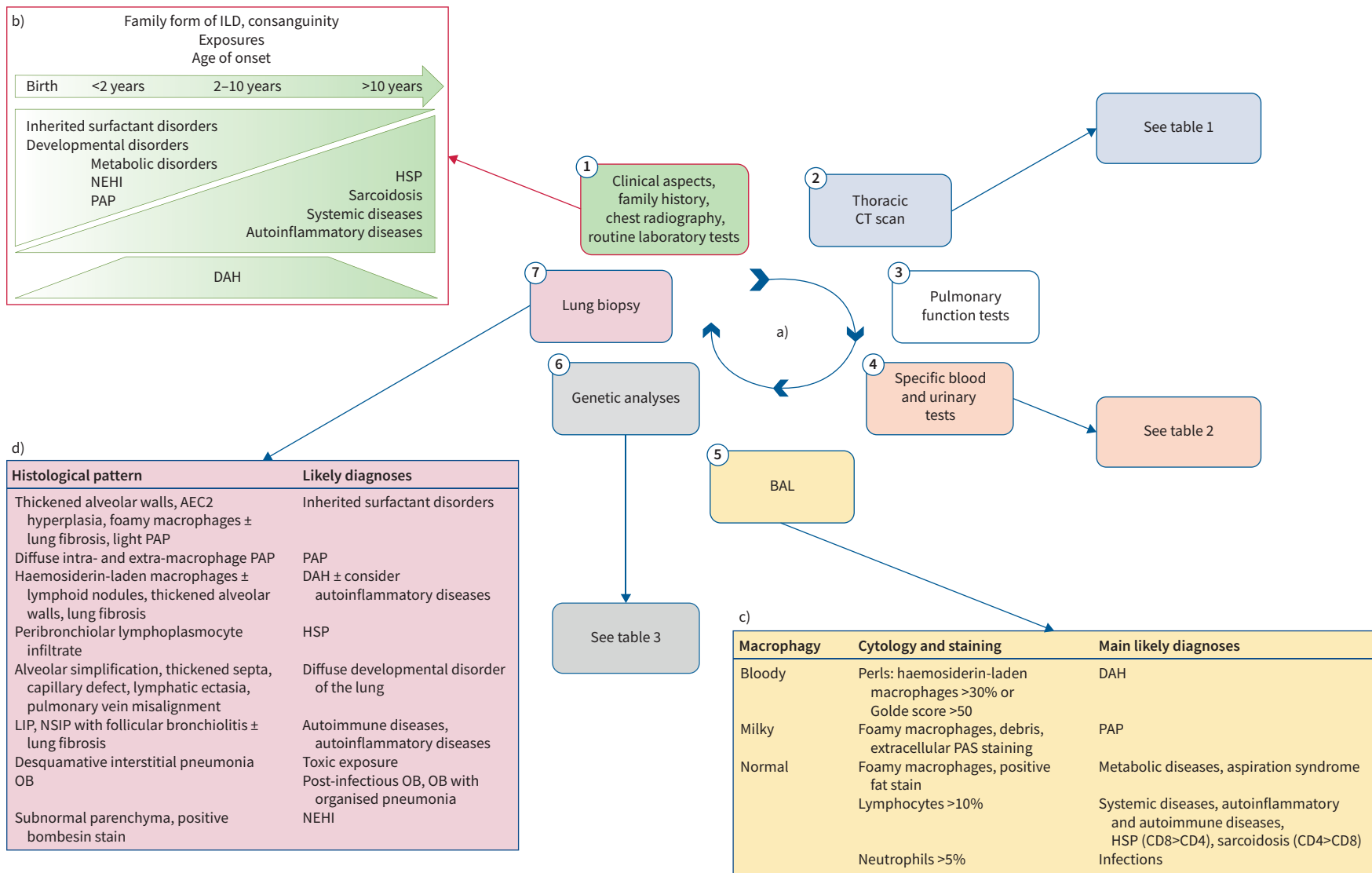
b)

Family form of ILD, consanguinity
Exposures
Age of onset

Birth   <2 years   2–10 years   >10 years

Inherited surfactant disorders
Developmental disorders
Metabolic disorders
NEHI
PAP

HSP
Sarcoidosis
Systemic diseases
Autoinflammatory diseases

DAH

1 Clinical aspects, family history, chest radiography, routine laboratory tests

2 Thoracic CT scan

See table 1

a)

7 Lung biopsy

3 Pulmonary function tests

6 Genetic analyses

4 Specific blood and urinary tests

See table 2

5 BAL

See table 3

d)

Histological pattern	Likely diagnoses
Thickened alveolar walls, AEC2 hyperplasia, foamy macrophages ± lung fibrosis, light PAP	Inherited surfactant disorders
Diffuse intra- and extra-macrophage PAP	PAP
Haemosiderin-laden macrophages ± lymphoid nodules, thickened alveolar walls, lung fibrosis	DAH ± consider autoinflammatory diseases
Peribronchiolar lymphoplasmocyte infiltrate	HSP
Alveolar simplification, thickened septa, capillary defect, lymphatic ectasia, pulmonary vein misalignment	Diffuse developmental disorder of the lung
LIP, NSIP with follicular bronchiolitis ± lung fibrosis	Autoimmune diseases, autoinflammatory diseases
Desquamative interstitial pneumonia	Toxic exposure
OB	Post-infectious OB, OB with organised pneumonia
Subnormal parenchyma, positive bombesin stain	NEHI

c)

Macrophagy	Cytology and staining	Main likely diagnoses
Bloody	Perls: haemosiderin-laden macrophages >30% or Golde score >50	DAH
Milky	Foamy macrophages, debris, extracellular PAS staining	PAP
Normal	Foamy macrophages, positive fat stain	Metabolic diseases, aspiration syndrome
	Lymphocytes >10%	Systemic diseases, autoinflammatory and autoimmune diseases, HSP (CD8>CD4), sarcoidosis (CD4>CD8)
	Neutrophils >5%	Infections

FIGURE 1 Investigations in childhood interstitial lung disease (chILD) diagnostic workup. a) The investigations are performed one after the other to reach a chILD aetiology. The first step is usually done by the general paediatrician (framed in red), and includes family history, clinical evaluation, chest radiography and routine laboratory tests. b) Age at onset is crucial information that helps to consider some aetiologies more than others. c) Bronchoalveolar lavage (BAL) eliminates infectious causes of ILD and provides clues to the most likely diagnoses. d) When thoracic computed tomography (CT) scan, specific blood tests, BAL and genetic analyses could not assess a diagnosis, lung biopsy is the last step of the diagnostic process, allowing to assess the most likely diagnoses in most cases. NEHI: neuroendocrine cell hyperplasia of infancy; PAP: pulmonary alveolar proteinosis; HSP: hypersensitivity pneumonitis; DAH: diffuse alveolar haemorrhage; PAS: Periodic acid–Schiff; AEC2: type 2 alveolar epithelial cells; LIP: lymphoid interstitial pneumonia; NSIP: non-specific interstitial pneumonia; OB: obliterans bronchiolitis.

Elementary lesions	Distribution	Suspected diagnoses
TABLE 1 Childhood interstitial lung disease diagnostic assessment based on computed tomography scan pattern		
GGO	Dense, diffuse	Inherited surfactant disorders
GGO, peripheral traction cysts	Diffuse	Inherited surfactant disorders
GGO, peripheral and/or parenchymal traction cysts, traction bronchiectasis, reticulations		Inherited surfactant disorders (older age); autoinflammatory disorders
Diffuse (sometimes ill-defined centrilobular) nodules, diffuse GGO ± alveolar consolidation	Patchy	Diffuse alveolar haemorrhage
GGO, cysts, honeycombing and reticulations	Peripheral	Connective tissue diseases, systemic and autoimmune diseases
GGO	Paramediastinal, paracardial, middle lobe, lingula (usual); others (aberrant)	Persistent tachypnoea of infancy/ neuroendocrine cell hyperplasia of infancy
GGO and air trapping	Centrilobular	Hypersensitivity pneumonitis
Reversed halo sign		Organising pneumonia
Crazy paving	More intense in lower lobes	Pulmonary alveolar proteinosis
Micronodules, hilar lymphadenopathies	Lymphatic distribution	Sarcoidosis
Centrilobular nodules	Diffuse	Hypersensitivity pneumonitis

GGO: ground-glass opacities.

ILD is often characterised by a restrictive ventilatory disorder, with a decrease in total lung capacity and vital capacity. Measurement of diffusing capacity of the lung for carbon monoxide (D_{LCO}) should be systematically performed according to the age of the child. Additionally, measurement of pulmonary compliance (an invasive examination which requires the placement of an oesophageal catheter) is done exceptionally to complete the evaluation [59]. In infants, PFTs can only be performed during sleep and therefore require the use of chloral pre-medication, the use of which is unauthorised in some countries and subject to signed informed consent in others. Between the ages of 3 and 6 years, PFTs require active cooperation (accepting at least a nose clip and a mouthpiece). After the age of 6–8 years, exploration approaches that of adults. Functional residual capacity is the most common measurement. Resistance, volumes and forced expiratory flows can complete the examination as association with an obstructive pathology is not exceptional.

Specific clinical laboratory tests

A number of blood and urinary tests are performed during the chILD workup. Most of them should be done only based on clinical orientation and age of onset. They are listed in table 2.

Bronchoalveolar lavage

As long as the medical condition of the patient allows it, flexible bronchoscopy with bronchoalveolar lavage (BAL) should be performed [61–65]. It allows cytological and microbiological analysis (bacteria, viral and fungi) of the alveolar fluid and collects the following information: volume and appearance of the fluid, cell count and staining (May–Grünwald Giemsa and Papanicolaou for cellular morphology, Perls to detect the presence of iron-containing cell samples, Periodic acid–Schiff (PAS) to detect polysaccharides such as glycogen, glycoproteins, glycolipids and mucins, and targeted staining (Ziehl and Grocott to detect mycobacteria and fungi, respectively)). A sample of BAL is kept (supernatant and cell pellets) for additional studies, in particular immunohistochemistry.

The BAL profile can guide the diagnosis, first with its macroscopic aspect and second with its cell count and specific staining (figure 1c). A global increase of the BAL cell count in the presence of a proven case of chILD and after exclusion of an infection may reflect alveolitis; however, the reference values for total BAL cell counts vary widely, so that this variable is not useful in everyday practice [61, 66]. The cytological examination makes it possible to search for pathogenic agents, viral inclusions, unusual macrophages (e.g. haemosiderin-laden macrophages and foamy macrophages), foreign bodies and abnormal cell populations [18]. Surfactant protein analysis by Western blot may be of interest but is not used routinely [67].

These results, together with those of the HRCT scan, allow a definite chILD diagnosis for some conditions, including hypersensitivity pneumonitis, pulmonary alveolar proteinosis, pulmonary haemorrhage and several infectious conditions such as *Pneumocystis jirovecii* infection [64].

TABLE 2 Biological investigations in childhood interstitial lung disease	
Investigation	**Indicates**
Haematology	
Complete blood count	
Reticulocytes	Anaemia and/or diffuse alveolar haemorrhage
Haemostasis	Anaemia and/or diffuse alveolar haemorrhage
Biochemistry	
Serum electrolytes, creatinine	
Liver enzymes	Hepatomegaly and/or pulmonary alveolar proteinosis
Thyroxine, thyroid-stimulating hormone	Surfactant disorder (*NKX2-1*)
Serum protein electrophoresis, sedimentation rate	Autoinflammatory/inflammatory disorder
Angiotensin converting enzyme	Sarcoidosis
Iron, ferritin	Anaemia and/or diffuse alveolar haemorrhage and inflammatory syndrome
Calcium, ionised calcium, phosphorous	Sarcoidosis
Lactate dehydrogenase	Alveolar lung injury
Proteinuria	Autoinflammatory/inflammatory disorder
Calciuria	Sarcoidosis
Ammonaemia	
Chromatography of blood and urine amino acids	Metabolic disorder, *e.g.* lysinuric proteinuria
Chromatography of urinary organic acids	
Serologies	
Epstein–Barr virus serology and viral load	
Cytomegalovirus serology and viral load	If subacute, neonatal or immune deficiency
HIV-1/HIV-2 serology and viral load	*Pneumocystis jirovecii*, immune deficiency
Mycoplasma pneumoniae serology and nasopharyngeal PCR	Subacute
Chlamydia pneumoniae serology and nasopharyngeal PCR	Subacute
Chlamydia trachomatis serology and nasopharyngeal PCR	Subacute in newborns
Ureaplasma urealyticum serology and nasopharyngeal PCR	
IgG precipitins	Hypersensitivity pneumonitis, farmer's lung, bird fancier's lung
Immunology	
Post-vaccinal serologies	Immune deficiency
IgG, IgA, IgM and IgG subclasses	Immune deficiency, autoinflammatory/inflammatory disorder
C3, C4, CH50	
Lymphocyte count/differential	
Circulating immune complexes	
Antinuclear antibodies	
ANCAc (PR3), ANCAp (MPO)	
Rheumatoid factor	
Anti-CCP	
Anti-cardiolipin	
Scleroderma, polymyositis and myositis antibodies (KU, PM-Scl75, TIF1γ, MDA-5, PM-Scl100, Ml2, KJ; anti-synthetase (PL7, PL12, OJ, centromere, SRP, JO1), smooth muscle, glomerular basement membrane)	Muscular, oesophageal and/or cutaneous involvement
GM-CSF auto-antibodies	Pulmonary alveolar proteinosis
IFN-α signature, IFN-α dosage	Immune deficiency, autoinflammatory/inflammatory disorder

ANCA: anti-neutrophil cytoplasmic antibody; PR3: proteinase 3; MPO: myeloperoxidase; CCP: cyclic citrullinated peptide; GM-CSF: granulocyte–macrophage colony-stimulating factor; IFN: interferon.

Cardiac ultrasound

Cardiac ultrasound must be carried out early and systematically as part of the severity assessment. It has three main purposes in the evaluation of chILD: 1) the search for pulmonary hypertension, which is an important prognostic factor and part of the Fan severity score items [52], but can also guide toward specific aetiologies such as diffuse developmental disorders of the lung and surfactant disorders in newborns [68], 2) the search for a left-sided heart pathology (to eliminate a differential diagnosis), and 3) the search for cardiac involvement in the context of a general illness (search for pericarditis and associated heart malformation).

Genetic tests

A genetic cause is currently identified in ~20% of patients with chILD (table 3) [11]. Genetic analysis is recommended for all paediatric patients with chronic ILD, whether sporadic or familial with no identified cause [69–72]. The analysis must be carried out by specialised genetics centres, and the detection of a genetic anomaly must always be explained to the patient and their family during genetic counselling consultation [71, 73].

The majority of patients in whom a genetic abnormality related to chILD is identified have a mutation in the genes encoding proteins of surfactant metabolism [25, 74–80]. Mutations in the *SFTPB* and *SFTPC* genes, encoding surfactant protein (SP)-B and SP-C, the surfactant transporter *ABCA3* (ATP binding cassette subfamily A member 3), and the transcription factor *NKX2-1* (or *TTF1* (thyroid transcription factor 1)) are most often implicated [81–85]. *SFTPA1* and *SFTPA2* (SP-A1 and SP-A2) and *FLNA* (filamin A) mutations have also very rarely been involved in chILD (but more often in adult ILD) [86–89]. If alveolar proteinosis is suspected, the genes *MARS* (methionyl-tRNA synthetase), particularly when elevated liver values are noted, and *CSF2RA* and *CSF2RB* (subunits α and β of the receptor) are studied [29, 90–92]. Other ARS (*FARSA, FARSB, YARS, IARS* and *LARS*) mutations have also been associated with rare cases of syndromic chILD [30, 31, 93–95].

Genetic abnormalities responsible for autoinflammatory diseases with autoimmunity have also been described in early chILD such as SAVI syndrome (STING-associated vasculitis of infancy) related to mutations in *TMEM173* (transmembrane protein 173) and COPA syndrome due to mutations in *COPA* (coatomer protein complex subunit α) [34–36, 96–98].

TABLE 3 Main genes and proteins currently implicated in childhood interstitial lung disease (chILD) groups, their mode of transmission and the associated phenotypes

Gene (protein)	Inheritance pattern	Phenotypes
Inherited surfactant disorders		
SFTPA1, SFTPA2	AD	Very rarely chILD, adult ILD and adenocarcinoma of the lung
SFTPB	AR	Neonatal respiratory distress ± PH
SFTPC	AD	Neonatal respiratory distress; ILD in infants or children, adults
ABCA3	AR	Neonatal respiratory distress ± PH; ILD in infants or children, adults
NKX2-1	AD	Brain–lung–thyroid syndrome
PAP		
MARS	AR	PAP; hepatomegaly with cholestasis, anaemia, neurological impairment
CSF2RA, CSF2RB	GR and AR	PAP (infants, children, adults)
GATA2	AR	Secondary PAP; immune deficiency with myelodysplasia
Autoinflammatory disorders		
TMEM173	AD	Early chILD with autoimmune and inflammatory disease ± joint and skin involvement
COPA	AD	Early chILD or DAH with autoimmune and inflammatory disease ± joint and kidney involvement
ZNFX1		chILD with severe viral infections, neurological symptoms, thrombotic microangiopathy
OAS1	AD	PAP with immunodeficiency and autoinflammation
Other chILD		
FLNA	GA and GD	chILD with emphysema; cardiac abnormalities, neurological impairment; girls>boys
NHLRC2	AR	FINCA
Diffuse abnormalities of lung development		
FOXF1	AD	chILD with PH; alveolar capillary dysplasia ± misalignment of pulmonary veins
TBX4, FGFR2	AD and AR	chILD with PH; acinar dysplasia
EIF2AK4	AR	chILD with PH; pulmonary haemangiomatosis; veno-occlusive disease

AR: autosomal recessive; AD: autosomal dominant; GR: gonosomal recessive; GD: gonosomal dominant; PH: pulmonary hypertension; PAP: pulmonary alveolar proteinosis; DAH: diffuse alveolar haemorrhage; FINCA: fibrosis, neurodegeneration and cerebral angiomatosis.

Finally, in the event of chILD secondary to a metabolic disease such as Niemann–Pick disease (*NPC1* and *NPC2*), mucopolysaccharidosis, alveolar microlithiasis (*SLC34A2*) or dibasic protein intolerance (*SLC7A7*), a targeted analysis of the involved genes is carried out.

Lung biopsy

The indications for lung biopsy are currently declining with the progress of genetic diagnostics. Previously considered as the gold standard for chILD diagnosis, it is now discussed as a last line of investigation [16, 17]. The lung biopsy is usually done as a surgical thoracoscopic or an open procedure depending on the centre's expertise and the child's age [99–101]. Transbronchial and transthoracic biopsies are not recommended for the diagnosis of chILD, as the samples obtained may be too small. Transbronchial biopsies are also limited by the size of the fibrescope, the biopsy forceps being larger than the operator channel of the smallest fibrescope. Histology analysis of the lung sample requires an expert pathologist as lung architecture has to be analysed in relation to the age of the child and the low number of lung biopsies reduces the experience of pathologists in centres without chILD expertise. If possible, a sample is dedicated to histology, another is fixed in glutaraldehyde buffer for electron microscopy and a frozen sample could be used for further somatic genetic analyses. Microscopic examination is carried out on standard stains (haematoxylin/eosin), special stains (Perls, PAS, Grocott, reticulin and Masson's Trichrome) and immunostaining (TTF-1, bombesin, surfactant proteins and vascular markers). The topography of the lesions is evaluated at low magnification and the elementary lesions are systematically analysed. The lesion profile must be correlated with the imaging data. This morphological analysis can identify specific histological profiles (figure 1d).

In the case of chILD with extrapulmonary involvement, the diagnosis may be obtained by biopsy of an organ that is easier to access than the lung. This is the case, for example, for sarcoidosis (salivary glands, adenopathy, liver, *etc.*) or dermatomyositis (skin, muscle, *etc.*) [102].

A stepwise approach to chILD diagnosis

As described in the previous section, the number of investigations required to approach a chILD diagnosis may be substantial and time consuming. Meanwhile, supporting treatment including oxygen therapy and nutritional supplementation are started [16, 17, 25]. The final diagnosis is often awaited to start more specific medications such as corticosteroids, azithromycin, hydroxychloroquine or anti-fibrosing therapies [103–112]. In chILD related to connective tissue diseases or autoinflammatory syndromes, immunosuppressive drugs such as mycophenolate mofetil, azathioprine, rituximab or Janus kinase inhibitors may be discussed [96, 97, 113–116].

Figure 2 attempts to schematise the step-by-step workup of chILD. Workup step 1 includes clinical evaluation, family history, routine biological tests, chest radiography and echocardiography. A normal chest radiograph does not rule out the presence of chILD. It may often allow suspecting a chILD diagnosis by the paediatrician. However, in all cases, the confirmation of the ILD diagnosis and its classification requires specialised expertise and sometimes non-routine testing. Workup step 2 is thus realised in expert centres and includes HRCT and further explorations (BAL and specific laboratory tests) that could allow assessing, in typical cases, the diagnoses of exposure-related ILD, hypersensitivity pneumonitis, systemic and autoimmune diseases, metabolic diseases, PTI/NEHI or diffuse alveolar haemorrhage. However, in most cases, genetic tests and/or lung biopsy are still required at that stage. Genetic tests can confirm the diagnosis of surfactant disorders, monogenic forms of pulmonary alveolar proteinosis, SAVI, COPA syndrome and others (table 3). Lung biopsy can confirm the diagnosis of NEHI, sarcoidosis and pulmonary interstitial glycogenosis. Diffuse developmental disorders usually require both investigations to precisely determine the stage of developmental arrest (acinar dysplasia, congenital alveolar dysplasia and alveolar capillary dysplasia with or without misalignment of pulmonary veins) and the extent of the lesions.

Multidisciplinary team approach and periodic re-investigation of "undefined chILD"

At each step of the diagnostic process, multidisciplinary team meetings are of major help to ascertain the diagnostic suspicion or its confirmation and discuss the management of the patient. In chILD, an assessment of the diagnosis reached by the referring team by a central peer review team did not confirm the diagnosis in 13% [11]. However, expert clinicians also need training in subcategorisation of the final diagnosis, as the overall inter-rater agreement was only 64% [11].

Undefined chILD?

While these multiple investigations are still pending, chILD with no identified cause may be labelled as a "working diagnosis of undefined ILD". However, when performed, a significant proportion of chILDs (up
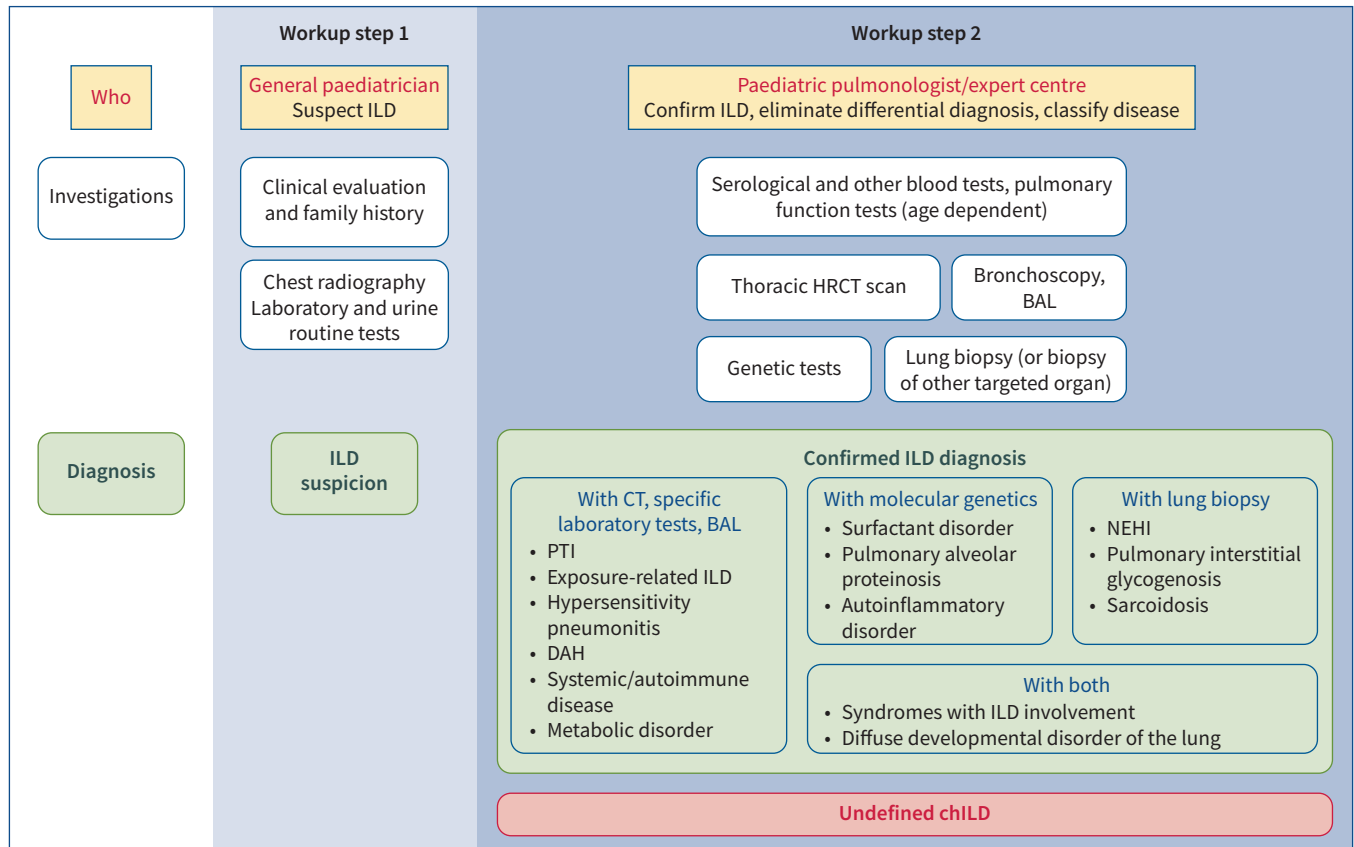
FIGURE 2 Stepwise approach to childhood interstitial lung disease (chILD) diagnosis. Workup step 1 represents the general paediatrician's role in chILD diagnostic workup. If the chILD suspicion is confirmed, the patient is referred to paediatric pulmonologists in expert centres for workup step 2: chILD confirmation and aetiological assessment. After specific investigations, usually including molecular analyses and/or lung biopsy, a chILD aetiology can be assessed in most cases. Other patients meet the definition of "undefined chILD". HRCT: high-resolution computed tomography; BAL: bronchoalveolar lavage; PTI: persistent tachypnoea of infancy; DAH: diffuse alveolar haemorrhage; NEHI: neuroendocrine cell hyperplasia of infancy.

to 12%) still remain unclassified. Based on the current (and evolving) knowledge, only those should be labelled as "undefined chILD".

It is indeed important to regularly reconsider undefined diagnoses regarding the evolution of the disease, the repetition of biological tests (especially autoimmune tests that can become positive with time) and the advances in chILD classifications in terms of new reported entities or new available molecular diagnoses. Some centres offer NGS panels, others propose WES systematically or after a negative NGS panel, and whole-genome sequencing is now available in some countries, blurring the line between diagnosis and research.

Points for clinical practice

- chILDs are rare and heterogeneous diseases with significant morbidity and mortality.
- The number of different chILD aetiologies is high and the diagnostic process requires a stepwise approach.
- The role of the general paediatrician facing a chILD suspicion is to question about the family and medical history and to initiate investigations (routine laboratory tests and chest radiography) before referring the patient rapidly to a specialised centre.
- Specific investigations, including CT scan, laboratory tests, BAL, PFTs, genetic testing and eventually lung biopsy, are run in expert centres and their results are discussed during multidisciplinary team meetings.
- This diagnostic workup, when complete, allows identification of a chILD aetiology in most cases.
- The remaining patients meet the definition of "undefined chILD". As medical progress is rapid, this diagnosis must be regularly reassessed.

Conclusions

The chILD diagnostic process can be simple and relatively short if a systematic two-step approach is followed. The role of the general paediatrician is crucial in untangling the personal and family medical history and the clinical signs, and in referring the patient to specialised centres when chILD is suspected. Even if easily accessible, the HRCT scan should be performed in a specialised centre to optimise its profitability. Lung biopsy is being dethroned by the fantastic progress in molecular diagnostics. However, a low number of expert geneticists may induce a prolonged delay in getting the results. Thus, for each patient, a multidisciplinary case-by-case discussion based on coherent algorithms (figure 2) could minimise chILD diagnostic delay and reduce the proportion of undefined chILD, allowing a maximum of these young patients to receive personalised treatments and to benefit from an improved prognosis.

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