Diffuse alveolar haemorrhage in children: an international multicentre study

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Shareable abstract (@ERSpublications)
Diffuse alveolar haemorrhage (DAH) in children is a rare condition. DAH is heterogeneous in its underlying cause, presentation and outcome, with high overall mortality. There is a broad variation in diagnostic workup and medical treatment across Europe. https://bit.ly/40KGoUh

Abstract

Background Paediatric diffuse alveolar haemorrhage (DAH) is a rare heterogeneous condition with limited knowledge on clinical presentation, treatment and outcome.
Methods A retrospective, descriptive multicentre follow-up study initiated from the European network for translational research in children's and adult interstitial lung disease (Cost Action CA16125) and chILD-EU CRC (the European Research Collaboration for Children's Interstitial Lung Disease). Inclusion criteria were DAH of any cause diagnosed before the age of 18 years.

Results Data of 124 patients from 26 centres (15 counties) were submitted, of whom 117 patients fulfilled the inclusion criteria. Diagnoses were idiopathic pulmonary haemosiderosis (n=35), DAH associated with autoimmune features (n=20), systemic and collagen disorders (n=18), immuno-allergic conditions (n=10), other childhood interstitial lung diseases (chILD) (n=5), autoinflammatory diseases (n=3), DAH secondary to other conditions (n=21) and nonspecified DAH (n=5). Median (IQR) age at onset was 5 (2.0–12.9) years. Most frequent clinical presentations were anaemia (87%), haemoptysis (42%), dyspnoea (35%) and cough (32%). Respiratory symptoms were absent in 23%. The most frequent medical treatment was systemic corticosteroids (93%), hydroxychloroquine (35%) and azathioprine (27%). Overall mortality was 13%. Long-term data demonstrated persistent abnormal radiology and a limited improvement in lung function.

Conclusions Paediatric DAH is highly heterogeneous regarding underlying causes and clinical presentation. The high mortality rate and number of patients with ongoing treatment years after onset of disease underline that DAH is a severe and often chronic condition. This large international study paves the way for further prospective clinical trials that will in the long term allow evidence-based treatment and follow-up recommendations to be determined.

Introduction

Diffuse alveolar haemorrhage (DAH) is a rare condition characterised by bleeding from the pulmonary vessels which may manifest as haemoptysis, anaemia, nonspecific respiratory symptoms, diffuse pulmonary infiltrates and hypoxaemic respiratory failure [1, 2]. Onset may occur at all ages.

DAH may be caused by a variety of conditions including capillaritis, autoinflammatory diseases, cardiovascular diseases, coagulopathies, immuno-allergic conditions or by medical treatment/drug-induced lung injury among other aetiologies [1]. In cases where no underlying primary condition or disease can be found despite an extensive workup, the condition may be denoted as idiopathic pulmonary haemosiderosis (IPH) [2]. The overall incidence of DAH is unknown. In children with IPH, the incidence has been estimated to be 0.24–1.23/million children per year, but the incidence is highly elevated in children with trisomy 21 [3–5]. The clinical presentation of DAH is very heterogeneous ranging from chronic cough and dyspnoea to haemoptysis, acute respiratory failure and severe anaemia [6–8]. No evidence-based treatment guidelines exist. If no underlying cause of the haemorrhage is identified, it is often treated with immunosuppressive therapy, especially high-dose pulsed corticosteroids (CCS), which have been used for the last 30 years with no scientific evidence for the efficacy [9, 10]. DAH outcome is poorly reported. The 5-year survival in IPH has been estimated to be as low as 67–84% [6–8, 11, 12].

The aim of this study was to collect a large international, clinical paediatric DAH dataset irrespective of the underlying cause. We proposed a re-assessment of the underlying condition with a description of clinical presentation, diagnostic workup, treatment and long-term outcome.

Methods

The study was designed as a retrospective, descriptive cross-sectional multicentre study. Members involved in the European network for translational research in children's and adult interstitial lung disease (Cost Action CA16125) and chILD-EU CRC (the European Research Collaboration for Children's Interstitial Lung Disease) were invited by e-mail to include potential patients from January 2020 to February 2021. A detailed datasheet regarding baseline characteristics, symptoms, diagnostic workup, pulmonary function, treatment, follow-up data and outcome was provided to all clinicians who agreed to participate in the study. Acceptable data sources were medical patient files and local patient registries.

Inclusion criteria

Children (0–17 years) diagnosed from January 1998 and onwards were included. The diagnosis needed to be confirmed by one or more of the following criteria: 1) high-resolution computed tomography (HRCT) findings consistent with DAH in combination with relevant symptoms (haemoptysis, respiratory distress, anaemia); 2) bronchoalveolar lavage (BAL) fluid positive for haemosiderin-laden macrophages (HLM) or macroscopic blood; and 3) lung biopsy with finding of HLM and/or description of bleeding (except procedure-related bleeding). Cases were excluded if pulmonary bleeding was caused by a focal bronchial lesion (*e.g.*, bronchiectasis, trauma, tumour, vascular malformations, tuberculosis).

All patient data were kept anonymised, and the data were shared according to regulations by local ethical authorities. Data were handled and secured according to Danish data protection and ethical regulations (JP-2021-566).

Definitions

Definitions of patient groups

Submitted cases were categorised into eight groups according to the childhood interstitial lung disease (chILD) diagnosis: 1) IPH (*i.e.*, no underlying condition or disease); 2) systemic and collagen disorders (systemic vasculitis and rheumatic diseases); 3) DAH associated with autoimmune features: patients who were not diagnosed with systemic or collagen disease, but who had elevated autoantibodies; 4) immuno-allergic conditions (Lane–Hamilton syndrome and pulmonary haemorrhage with cow's milk IgG – previously labelled as Heiner syndrome [13]); 5) autoinflammatory diseases; 6) other chILD; 7) DAH secondary to other conditions (including external factors such as medication and toxication); and 8) nonspecified DAH diagnosis.

Definition of follow-up

Clinical follow-up status was categorised as: healthy; chronic clinically stable (a chronic condition with impaired lung function and/or persistent abnormal radiology but off medication for DAH); active clinically stable (under current treatment); unstable (symptomatic and/or impaired lung function despite treatment); alive but in an unknown condition; unknown; dead.

Lung function

Results from available spirometry, lung volumes (total lung capacity) and diffusing capacity of the lung for carbon monoxide were reported. Only measurements performed no later than 1 month after diagnosis were included for assessment of initial lung function measurement. Parameters were reported as % predicted. Spirometry data were used to classify lung function impairment as restrictive (forced expiratory volume in 1 s (FEV_1) <80%, forced vital capacity (FVC) <80% and FEV_1/FVC >80%) or obstructive (FEV_1 <80% and FEV_1/FVC <80%).

Genetic testing

Results from any genetic analysis were included ranging from targeted single gene analysis to whole genome sequencing depending on local practice.

Statistics

Data were presented as median (IQR); median (range) was used when number of observations was <5. Kruskal–Wallis test and multiple pairwise comparison were used to compare duration of medical treatment between diagnostic groups. Paired t-tests were used to compare baseline and follow-up pulmonary function parameters in each patient group. A p-value <0.05 was considered significant. If data concerning symptoms, clinical examinations and treatment were omitted in a patient, they were treated as missing and not included in the calculation of prevalence. Statistical analyses were performed using RStudio (RStudio, Boston, MA, USA) version 1.4.1717.

Results

Clinicians from 26 centres in 15 countries submitted 124 patient cases of whom 117 were included. Six patients were excluded due to missing information regarding diagnostic workup and one was miscategorised. Detailed information on contributing centres is available in supplementary table S1.

Diagnosis

Figure 1 shows the diagnosis repartition into eight subgroups: 22% (n=10) of patients were reallocated from IPH to other groups. Further details of diagnosis and baseline characteristics are presented in tables 1 and 2.

Clinical presentation

The median (IQR) age at presentation was 5 (2–12.9) years with a median (IQR) diagnostic delay of 2 (0–12) months (table 2). The sex distribution was 57% females (ranging from 20% to 70%). Overall, anaemia (87%), haemoptysis (42%), dyspnoea (35%) and cough (32%) were the most frequent clinical symptoms at initial presentation with only minor differences between the subgroups (figure 2). In 20 (17%) haemoptysis was the only respiratory symptom, while respiratory symptoms were absent in 27 (23%) at initial presentation. Of those without respiratory symptoms, anaemia was described in 21 (78%) at initial presentation. Diagnostic delay was not different between patients with and without respiratory symptoms at initial presentation (p=0.67).

Clinical workup

HRCT (94%), BAL (85%) and echocardiography (80%) were the most frequently performed diagnostic procedures (table 3). Lung biopsy was performed in 49 (42%) and genetic testing was performed in 52
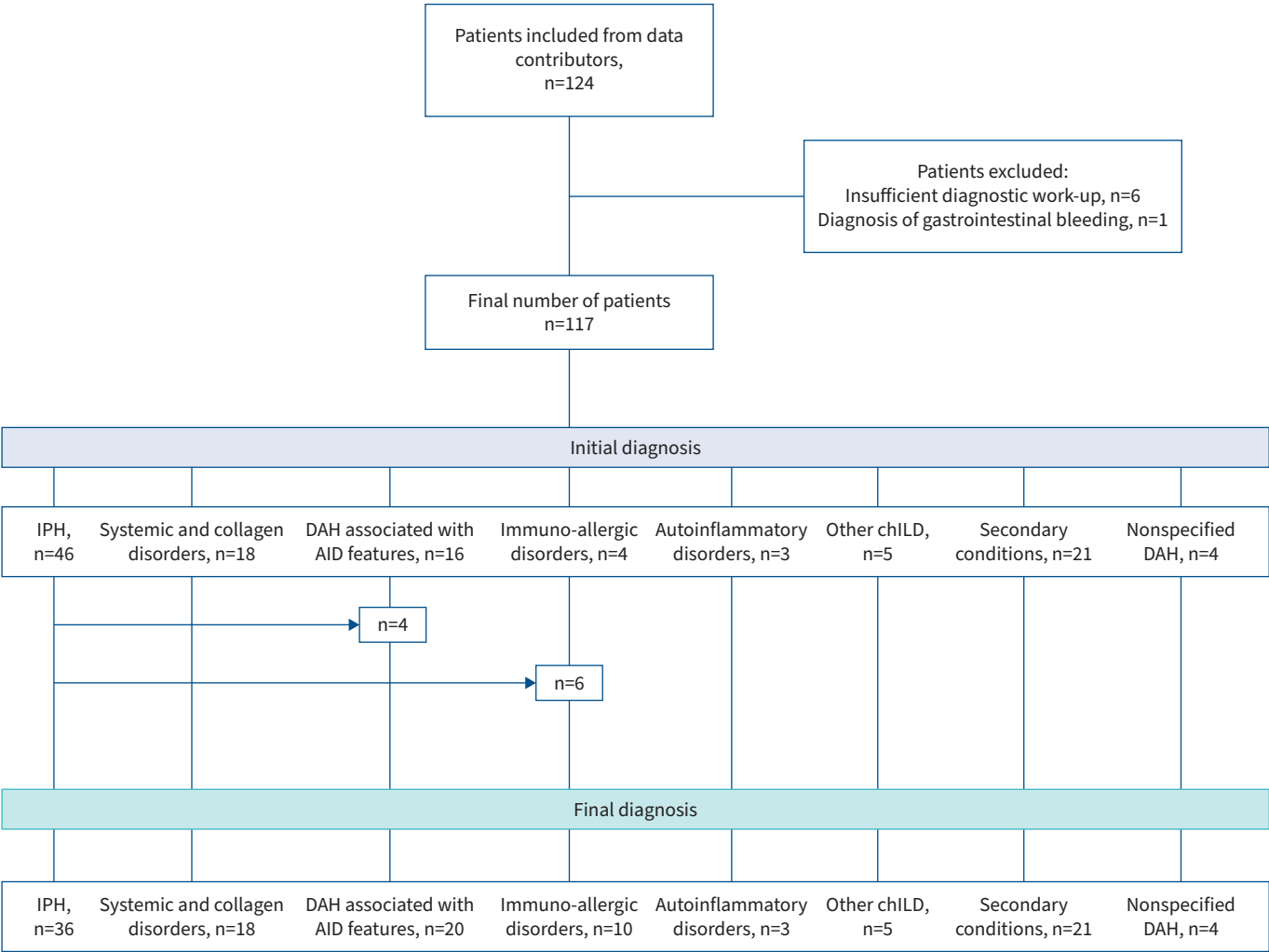
FIGURE 1 Inclusion process and distribution of patients with diffuse alveolar haemorrhage (DAH). IPH: idiopathic pulmonary haemosiderosis; AID: autoimmune disease; chILD: childhood interstitial lung disease.

(44%). Rheumatological workup was performed on 103 patients (88%) of whom 41 (40%) tested positive for one or more autoantibodies. Anti-transglutaminase IgA and cow's milk IgG were elevated in 17% and 14% of those who had been tested, respectively. More detailed information on diagnostic workup performed in each subgroup is available in supplementary tables S2 and S3). Pulmonary function data were available in 36 (68%) of 53 patients 6 years of age or older. Pulmonary function data demonstrated a restrictive impairment, obstructive impairment or normal pulmonary function in 22 (61%), 4 (11%) and 11 (31%) patients, respectively (supplementary table S4).

Treatment

Systemic corticosteroids (sCCS) was the most frequently reported medical treatment (93% of patients), administered as intravenous (*i.v.*) high-dose pulse methylprednisolone (15–30 mg·kg^{-1} for 3 days) (69%) or oral prednisolone (78%). 72% of all patients received sCCS treatment with both *i.v.* pulses and oral prednisolone. Monotherapy with sCCS was used in 43% of patients, while sCCS were supplemented with either hydroxychloroquine, azathioprine or both in 23%, 14% and 12% of patients, respectively (figure 3). Other frequently administered medical treatments included mycophenolate mofetil, cyclosporine, cyclophosphamide and rituximab as the most frequent (supplementary table S5). Two patients (a patient diagnosed with IPH and a patient with STAT3 mutation) required lung transplantation.

The median (IQR) treatment duration of *i.v.* CCS pulses was 3 (1–9) months for all DAH patients whereas oral prednisolone, hydroxychloroquine and azathioprine treatment continued for 1.3 (0.5–3.5),

TABLE 1 Diagnosis in patients with diffuse alveolar haemorrhage (DAH)

Diagnosis	
All DAH patients	**117**
Idiopathic pulmonary haemosiderosis	**36**
DAH associated with autoimmune features	**20**
Nonspecific but ANA positive	8
Nonspecific ANCA-associated vasculitis	9
Nonspecific but SMA positive	1
Nonspecific but positive rheumatoid factor	2
Systemic and collagen disorders	**18**
Granulomatosis with polyangiitis	6
Microscopic polyangiitis	3
Polyarthritis nodosum	1
Churg–Strauss syndrome	2
Anti-glomerular basement membrane disease	3
Systemic lupus erythematosus	1
Antiphospholipid syndrome	1
Membranous glomerulopathy	1
Immuno-allergic disorders	**10**
Pulmonary haemorrhage with cow's milk antibody (IgG)	4
Lane–Hamilton syndrome	6
Other chILD	**5**
Pulmonary interstitial glycogenosis	2
Postinfectious bronchiolitis obliterans	1
SP-C disorder	1
Nonspecific lung fibrosis	1
Autoinflammatory diseases	**3**
COPA syndrome	2
STAT-3 mutation	1
Secondary to other conditions	**21**
Infectious	7
Malignancy	1
Cardiovascular disease (including pulmonary arterial hypertension)	4
Lung damage due to exogenous toxicity	2
Coagulopathy	1
Bone marrow transplant-related lung injury	1
Transfusion-related lung injury	1
Cantu syndrome with impaired lung growth and pulmonary hypertension	1
Wilson disease with acute liver failure	1
Familiar cholestasis type 1: post-liver transplantation complication	1
Wiskott–Aldrich syndrome with severe septic shock and coagulopathy	1
Nonspecified DAH diagnosis	**4**

ANA: antinuclear antibodies; ANCA: anti-neutrophil cytoplasm antibodies; SMA: smooth-muscle antibodies; chILD: childhood interstitial lung disease; SP-C: surfactant protein C; COPA: COPI coat complex subunit α; STAT-3: signal transducer and activator of transcription 3.

TABLE 2 Baseline characteristics and age at debut and diagnosis

Diagnosis	Subjects n	Females	Age at debut	Age at diagnosis	Diagnostic delay	Trisomy 21
All DAH patients	117	67 (57)	5 (2–12.9)	6.1 (3–13)	2 (0–12)	7 (6)
IPH	36	21 (60)	3.0 (1.3–5.0)	4.2 (3.0–7.4)	10 (2–21)	3 (8)
DAH associated with autoimmune features	20	13 (65)	4.5 (1.8–11)	4.8 (2.3–11)	2 (0–11)	0
Systemic and collagen disorders	18	12 (67)	14.2 (12.1–15.4)	14.2 (12.6–15.5)	0 (0–3)	1 (6)
Immuno-allergic disorders	10	7 (70)	5.1 (2.5–11.6)	5.2 (3.4–12.7)	2 (1–4)	0 (0)
Other chILD	5	2 (40)	1.0 (0–5.8)	1.3 (0–5.8)	0 (0–0)	0 (0)
Autoinflammatory diseases	3	2 (67)	2.0 (2.0–2.1)	5.0 (4.0–9.0)	35 (24–84)	0 (0)
Secondary to other conditions	21	9 (43)	7.4 (1.2–14.1)	7.6 (3.0–15.1)	2 (0–9)	2 (10)
Nonspecified DAH diagnosis	4	1 (25)	12 (6.8–15,3)	10.3 (8.6–15.8)	1 (0–1)	1 (25)

Data are presented as n (%) or median (IQR), unless otherwise stated. DAH: diffuse alveolar haemorrhage; IPH: idiopathic pulmonary haemosiderosis; chILD: childhood interstitial lung disease.
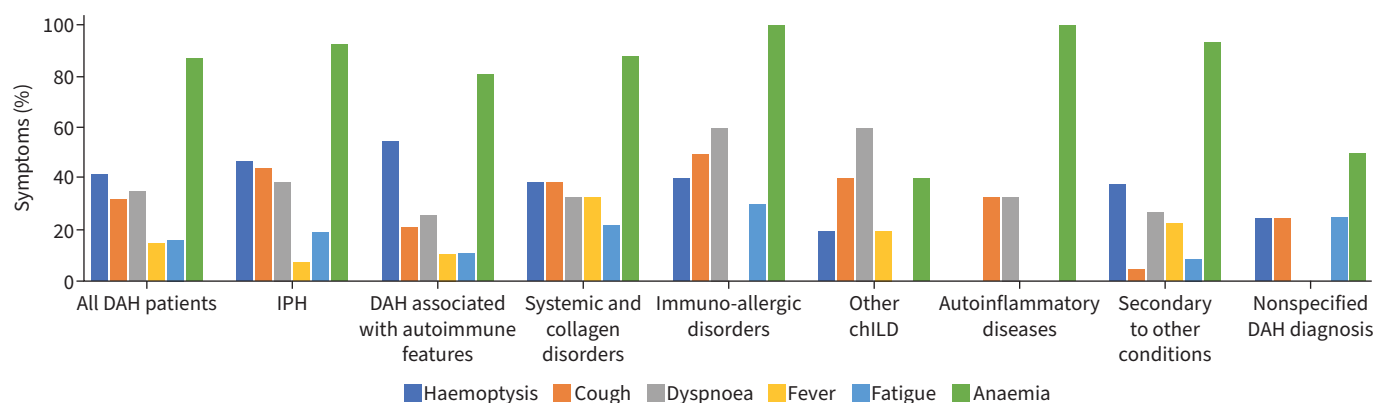
FIGURE 2 Symptoms and clinical presentation. The proportion of symptoms in each subgroup reported as percentages. Note: one patient may have more than one symptom. DAH: diffuse alveolar haemorrhage; IPH: idiopathic pulmonary haemosiderosis; chILD; childhood interstitial lung disease.

2.8 (1.5–4.1) and 1.7 (0.5–3.2) years, respectively. No significant difference in length of treatment was found between the largest subgroups (IPH, systemic and collagen disorders, immune-allergic conditions and secondary to other conditions).

Follow-up and outcome

The median (IQR) follow-up period was 3.2 (1.2–7.0) years from diagnosis. In patients with both baseline and long-term follow-up lung function data (n=44), there was no significant change except in FVC and total lung capacity (TLC) with an improvement of 21% and 17% (p<0.001), respectively. Follow-up HRCT or radiograph was performed in 101 patients (86%) with a median (IQR) time of 2.5 (1.0–7.0) years after the baseline radiology (figure 4). Persisting abnormal radiology was recorded in 61/101 (60%) of patients (50% of patients otherwise considered healthy; 73% of patients considered as chronic but not on medical treatment and 80% of patients with ongoing treatment) (Table S6). Ground glass opacity (n=39), interstitial thickening (n=9) and fibrosis (n=7) were the most frequent findings described in follow-up HRCT/chest radiograph (Table S6).

Outcome data were available for 90%, of whom 86% were still alive: 19% were considered healthy, 16% were chronic clinical stable; 31% were active clinical stable and 12% with ongoing medical treatment and were considered unstable. 15 (13%) patients died (figure 5 and supplementary table S7). Outcome in patients who tested positive for antinuclear antibodies (ANA) was not reported as either unstable or dead in any of the patients (n=17) (data not presented). The sex distribution of the 15 patients who died was 54% females. The median (IQR) time from debut of symptoms to death was 1.8 (1.3–4.0) years. The most frequent causes of death were acute bleeding (n=4), pulmonary infection (n=4), chronic respiratory failure (n=3) and complications of lung transplantation (n=2). Lung transplantation was only performed in these two patients of whom one was diagnosed with IPH and the other with STAT-3 mutation (supplementary table S7).

Discussion

We report, to the best of our knowledge, the largest published multicentre study to date covering the entire spectrum from clinical presentation to treatment and outcome in children and adolescents diagnosed with DAH.

Clinical presentation

We showed that it is not possible to differentiate between the different subgroups of DAH by their clinical presentation or disease progression. Age at onset of disease was later in childhood in patients diagnosed with systemic and collagen conditions compared to the other subgroups. Anaemia was the most frequent clinical presentation, while respiratory symptoms (haemoptysis, cough, dyspnoea) only presented in approximately three-quarters of the entire cohort thus illustrating a diagnostic pitfall. The prevalence of respiratory symptoms in previously published IPH cohorts is comparable to our study, while the prevalence is higher in cohorts of DAH in childhood related to autoimmune diseases [6, 8, 14–16].

TABLE 3 Diagnostic workup in 117 patients with diffuse alveolar haemorrhage (DAH)		
	Patients who were tested, n (%)	Of those examined who tested positive, n (%)
HRCT	110 (94)	
Ground-glass opacity		78 (71)
BAL	100 (85)	
HLM		74 (74)
Fresh bleeding		28 (28)
Findings not described		14 (14)
Lung biopsy	49 (42)	
HLM		25 (51)
Vasculitis/capillaritis		5 (10)
Fibrosis		8 (16)
Echocardiography	94 (80)	
PAH		11 (12)
Genetic analysis (WES/NGS/not described)	52 (44) (1/8/43)	
COPA		2[#]
STAT-3		1[#]
APT7B		1[#]
SFTPC		1[#]
CYBB		1[#]
NKX2.1		2[#]
TBX4		1[#]
Other[¶]		7[#]
COPA negative		12[#]
Autoantibodies	103 (88)	
ANCA	100 (85)	17 (17)
ANA	96 (82)	17 (18)
Anti-double-stranded DNA and anti-SMA	74 (63)	7 (9)
RF, IgM	66 (56)	10 (15)
AEA	51 (44)	5 (10)
Specific immunoglobulins		
ATA, IgA	69 (59)	12 (17)
ATA, IgG	55 (47)	4 (7)
Cow's milk, IgG	37 (32)	6 (14)

HRCT: high-resolution computed tomography; BAL: bronchoalveolar lavage; HLM: haemosiderin-laden macrophage; PAH: pulmonary arterial hypertension; *COPA*: COPI coat complex subunit α; *STAT-3*: signal transducer and activator of transcription 3; *ATP7B*: ATPase cobber transporting β; *SFTPC*: surfactant protein C; *CYBB*: cytochrome B-245 β chain; *NKX2.1*: NK Homeobox 1; *TBX4*: T-box transcription factor 4; ANCA: antineutrophilic cytoplasmic antibodies; ANA: antinuclear antibodies; SMA: smooth muscle antibodies; RF: rheumatoid factor; AEA: anti-endomysium antibodies; ATA: anti-transglutaminase. [#]: human leukocyte antigen DQ α 1 (HLA DQA1), HLA DQB1 and HLA DQ β 1, n=1. 22q11 (DiGeorge's syndrome), n=1. PFIC-1 (progressive familial intrahepatic cholestasis 1), n=1). WASp (Wiscott–Aldrich Syndrome), n=1. FLT3-ITD (fms-like tyrosine kinase 3 internal tandem duplication), n=1. WT1 (Wilms' tumour 1), n=1. Heterozygote for *CSF2RB* (colony stimulating factor 2 receptor subunit β) (pulmonary alveolar proteinosis), n=1. [¶]: percentage not reported since the number of patients who had been tested for each condition/mutation is uncertain.

Trisomy 21 is thought to be associated with IPH, although the literature is conflicting with 1 out of 108 (0.9%) to 9 out of 34 (26%) reported frequencies [7, 17]. In our cohort 3 (8%) patients were diagnosed with trisomy 21. This increased risk of IPH in patients with trisomy 21 may be associated with the increased occurrence of conditions like autoimmune diseases, pulmonary hypertension and leukaemia [18, 19].

Diagnostic workup

The use of diagnostic workup (HRCT, BAL, lung biopsy) was overall consistent with an international survey responded to by 88 physicians caring for 274 paediatric IPH patients [20]. However, lung biopsy was performed more frequently than in our cohort (52% *versus* 42%). Indication for lung biopsy in DAH is not well defined; however, for a full workup and to reveal capillaritis, a lung biopsy is required [1]. Only 43% of the IPH patients had lung biopsy performed, and the potential underlying pathology (*e.g.*, capillaritis) may therefore be underdiagnosed in the remaining patients. In 13% of patients who had BAL performed no HLM were found, although in ~50% of these fresh bleeding was described. HLM is
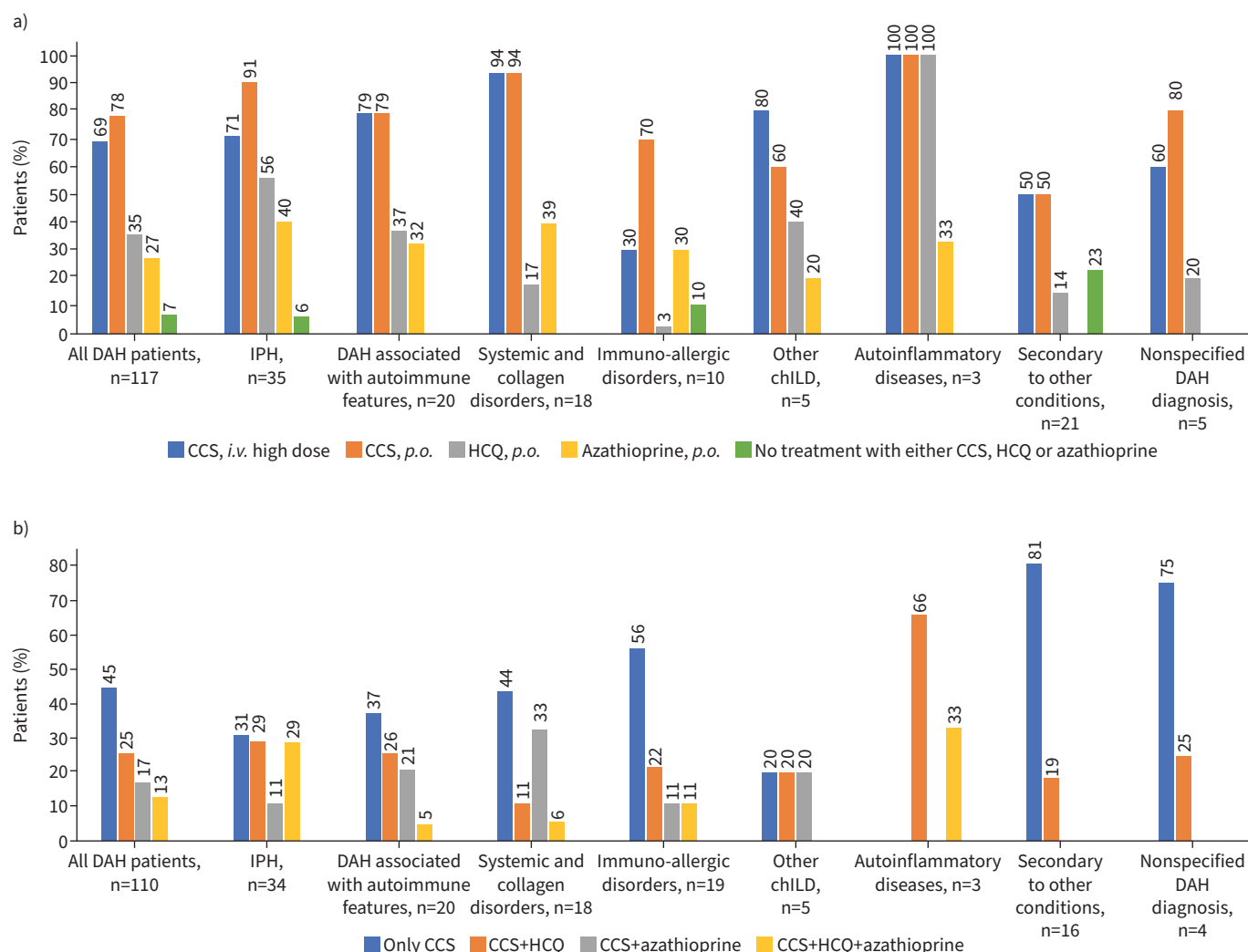
FIGURE 3 Medical treatment. a) Percentage of patients in each subgroup treated with systemic corticosteroids (CCS), hydroxychloroquine (HCQ), azathioprine or none of the above. b) Percentage of patients receiving different combination of treatment in each subgroup. Only patients who have received treatment are included. DAH: diffuse alveolar haemorrhage; IPH: idiopathic pulmonary haemosiderosis; chILD: childhood interstitial lung disease.

considered an important indicator of DAH, but the absence of HLM in BAL fluid cannot rule out DAH especially when BAL is performed between episodes of bleeding or in an unaffected lobe [21, 22]. Data on pulmonary functions tests were only available in a limited number of patients, which may be explained by the fact that only 45% of the included patients were 6 years of age or older at diagnosis. As expected, a restrictive pattern was mainly demonstrated with a large variation between the patients. Except for the DAH associated with autoimmune features, autoinflammatory diseases and chILD subgroups, lung function was not severely impaired [23–25].

Diagnosis

Most of the larger published IPH cohorts have included patients with Lane–Hamilton syndrome, pulmonary haemorrhage with cow's milk antibody and patients who tested positive for autoantibodies [6, 8, 14]. In this study, we chose to reshuffle these patients (~22%) from the IPH cohort into more specific subgroups (immune-allergic conditions and DAH associated with autoimmune features). This number is comparable to the prevalence of autoantibodies in IPH (26.4%) described in a literature review of published studies from 1981 to 2021 [26]. Such differences in workup and definitions of subgroups make it difficult to compare the different studies. A newly published retrospective study of all DAH patients in the European chILD-EU register has resulted in the suggestion of a diagnostic approach to DAH in children and descriptive clusters to guide further research [27].
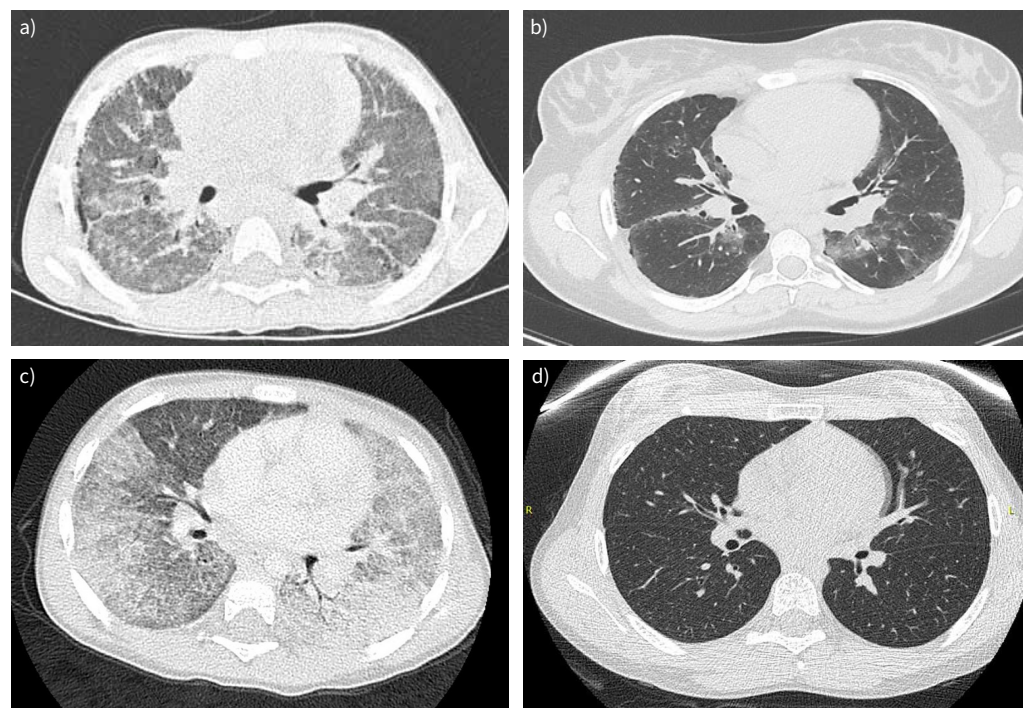
FIGURE 4 Radiology and follow-up. High-resolution computed tomography (HRCT) of two patients before and after medical treatment. a) Pre-treatment HRCT of a female patient with presentation of anaemia and recurrent lower respiratory infections with first appearance at age 2.1 years. HRCT was performed at time of diagnosis at age 4.8 years. b) Latest HRCT performed on same patient after 11 years of treatment with pulse methylprednisolone and hydroxychloroquine due to several relapses. HRCT shows incomplete response to treatment. Patient is still treated with pulses of methylprednisolone and hydroxychloroquine. c) Pre-treatment HRCT of female patient with presentation of cough, dyspnoea, tachypnoea, cyanosis, haemoptysis and recurrent lower airway infection at age 6 months. HRCT was performed at time of diagnosis at 2 years of age. d) Most recent HRCT after 7 years of treatment with pulse methylprednisolone and azathioprine due to several relapses. HRCT shows almost complete regression of pathological changes. Patient is out of treatment and considered healthy.

A better understanding of the pathogenesis may potentially lead to a more accurate diagnosis. A paper by SAHA *et al.* [28] reviews different hypotheses of IPH pathogenesis and suggests that bleeding in IPH is caused by bioactive proteins such as histamine, eosinophilic cationic protein and possibly vascular endothelial growth factor. Further studies are needed to understand the pathogenesis of IPH fully.

Treatment

There are no evidence-based guidelines or consensus on treatment regimens in paediatric DAH. Hence, treatment is based on local expert opinions and is largely the same treatment as that used against chILD in general. The mainstay of treatment was sCCS (92%) independent of the underlying condition. sCCS are used in other groups of chILD with no clear evidence as to whether *i.v.* high-dose pulses are more effective than oral daily administration. However, fewer side-effects from sCCS pulse treatment have been reported [9, 23]. Hydroxychloroquine and azathioprine were frequently combined with sCCS and are known to have an immunomodulatory effect, and the former especially has been used off-label in chILD for decades. In a literature review of 83 published chILD cases treated with hydroxychloroquine, the drug was found to be well tolerated in most cases and clinical condition improved in 35 patients [29]. A prospective randomised phase 2 trial of hydroxychloroquine treatment in chILD of all causes has recently been published. The study, which included two patients with IPH among a total of 35 patients, did not demonstrate any effect of treatment on oxygenation or respiratory rate but found that the treatment was well tolerated [30]. The use of azathioprine in pulmonary diseases is even less well documented in the literature and its role in the treatment of chILD and DAH is unknown. All patients in our study who were treated with azathioprine were additionally treated with sCCS alone or in combination with hydroxychloroquine suggesting that azathioprine is not used as a first-line treatment. Combination
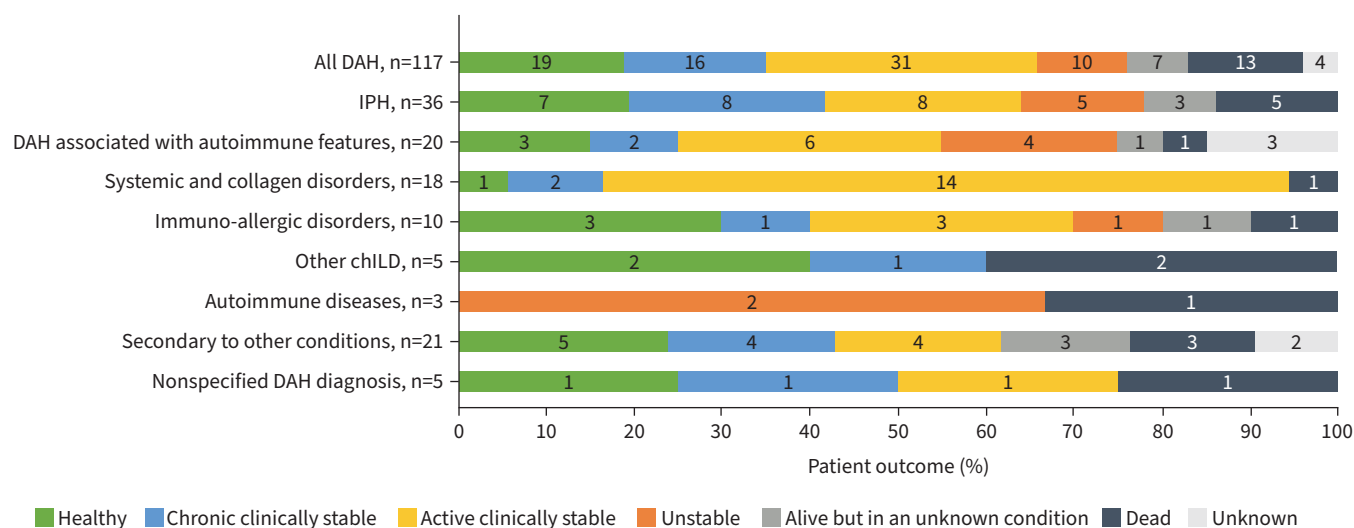
FIGURE 5 Patient outcome in the DAH cohort and the different subgroups presented as percentage: healthy; chronic clinically stable (chronic condition with impaired lung function and/or persistent abnormal radiology but off medication for DAH); active clinically stable (under current treatment); unstable (symptomatic and/or impaired lung function despite treatment); alive but unknown condition; unknown; and dead. DAH: diffuse alveolar haemorrhage; IPH: idiopathic pulmonary haemosiderosis; chILD; childhood interstitial lung disease.

treatment with sCCS, hydroxychloroquine and azathioprine was more frequently seen in patients with IPH and autoinflammatory disease, compared to the other patient subgroups. This may not only reflect the complexity of treating these conditions but also that severe progression sometimes requires more treatment. Rituximab was sporadically used (mainly in systemic and autoimmune conditions) but the use of biological treatment needs still to be clarified in other subtypes of DAH. Our cohort was overall treated for several years, which underlines the chronicity of these conditions. Importantly, it underlines that no consensus exists on gradual dose reduction or when to change to other medicine or stop medical treatment.

Follow-up

Knowledge of long-term prognosis in DAH is very limited and mainly derived from IPH. No consensus exists regarding the appropriate monitoring and follow-up in DAH, which is reflected by the heterogeneity of our data from the different centres. In other smaller IPH cohorts mean or median follow-up period was 2.3–5.5 years [6, 7, 10, 14, 31]. Variations in length of follow-up may partly be explained by disease severity but also by differences in healthcare systems. Our data indicate that lung function other than FVC and TLC did not improve, and imaging demonstrated that persistent structural abnormalities were common (64%). From our data, it is not clear whether lung function or radiology may be significantly improved by longer or more aggressive treatment or whether treatment mainly should be based on clinical symptoms.

Outcome

Very few patients (19%) were considered completely healthy with no significant differences among the subgroups at the time of observation. In DAH secondary to other conditions a larger proportion were considered healthy, which may be expected because of a more specific and treatable underlying cause of DAH. DAH regardless of the underlying condition is a potentially serious condition carrying considerable mortality. The mortality rate of 14% in the IPH group is comparable to an American and Turkish study in which 17% and 13% of patients had died [14, 32], but high compared to smaller IPH cohort studies: the French RespiRare cohort and an Indian cohort in which 1 out of 25 (4%) and 2 out of 26 (8%) had a fatal outcome, respectively [6, 8, 31]. In the Turkish follow-up study patients with an unfavourable IPH outcome (no response to steroids and requirement for further immunosuppressant agents or development of pulmonary hypertension/fibrosis/death) were compared to those with a favourable outcome [32]. The authors found a significantly higher number of patients who tested ANA positive in the group of patients with an unfavourable outcome (seven *versus* one). This was not the case in our study, but that might be due to different definitions of outcomes. Follow-up studies in patients with DAH caused by autoimmune disease have reported a relatively higher mortality compared to IPH patients in the literature [16, 33]. The mortality was lower among patients with autoimmune diseases and DAH associated with autoimmune features (6% and 5%) in our study compared to the entire group of DAH patients (13%). No clear

explanations for the difference in mortality of DAH exist. One may speculate that treatment guidelines including biological treatment are more available and evident for systemic and autoimmune conditions. A literature review investigated outcome in patients with IPH who received lung transplantation [34]. The review revealed four causes of adult females of whom two developed recurrence of lung bleeding post-transplantation. This may suggest a systemic cause of lung damage. Out of 117 patients in our study, only two received lung transplantation, of whom one was diagnosed with IPH and who subsequently died from acute rejection of the new lungs.

Strengths and limitations

The strength of this study was primarily the large international data set on a rare condition provided by the patients' treating paediatric pulmonologists, which likely increased the data reliability. Furthermore, we included a very broad spectrum of clinical data from initial presentation to long-term outcome. A limitation in this study design is the potential for acquisition bias since only physicians involved in the European networks Cost Action CA16125 and chILD-EU CRC were invited to participate.

The study is limited by the retrospective design with a long inclusion period which may have resulted in missing data. In addition, some of the DAH subgroups included very few patients due to the rarity of the disease. Results and statistical analyses on such small groups should always be interpreted with caution. As pulmonary function data were collected over three decades, variations in reference material used may be expected, but most tertiary centres are likely to have used the Global Lung Function Initiative (GLI) as a standard for the last decade [35]. Even if the level of pulmonary function may be influenced by the reference material used at the different centres, we do not expect these differences to be of significant importance for our results and lung function trends [36]. No standard panel for genetic testing has been reported since without doubt this will have varied through time and between centres. The number of genetic mutations may be higher than reported here due to insufficient testing compared to an ever-increasing standard.

The categorisation of patients is challenged by the time span of three decades: increased knowledge of different histological features from lung biopsies and significant improved genetic testing might have specified more underlying diseases from the IPH group.

Conclusion

This is, to our knowledge, the largest published multicentre study to report clinical data and prognosis in children with DAH. Importantly, many patients had no respiratory complaints at the initial presentation, and anaemia proved to be the most prominent symptom. Therefore, DAH must be considered in cases of unexplained anaemia even without notable respiratory symptoms. In combination with lung function, HRCT should be performed early in the workup process. Long-term data revealed that DAH is a severe condition with considerable mortality. In many cases, we documented persistent structural pulmonary abnormalities and a lack of significant improvement in lung function, evolving into a chronic, stable condition. The variation in diagnostic workup (BAL, echocardiography, lung biopsy and laboratory workup), treatment and time of follow-up demonstrated in this study supports the need for international consensus through prospective multicentre studies. This large international study paves the way for further prospective clinical trials that will in the long term allow evidence-based treatment and follow-up recommendations to be determined.

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