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Early View

Original research article

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Diffuse alveolar haemorrhage in children: an international multicentre study

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Introduction:

Diffuse alveolar haemorrhage (DAH) is a rare condition characterised by bleeding from the pulmonary vessels which may manifest as haemoptysis, anaemia, non-specific respiratory symptoms, diffuse pulmonary infiltrates and hypoxaemic respiratory failure (1,2). Onset may occur in 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 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 heterogenous 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, treatment with immunosuppressive therapy, especially high-dose pulsed corticosteroids, have been used for the last 30 years with no scientific evidence (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. The diagnosis needed to be confirmed by one or more of the following criteria: 1. HRCT findings consistent with DAH in combination with relevant symptoms (haemoptysis, respiratory distress, anaemia). 2. BAL fluid positive for haemosiderin-laden macrophages (HLM) or macroscopic blood; 2. Lung biopsy with finding of HLM and/or description of bleeding (except procedure-related bleeding). Exclusion criteria: 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 was handled and secured according to Danish data protection and ethical regulations (JP-2021-566).

Definitions

1. Definitions of patient groups

Submitted cases were categorised into eight groups according to the 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 as medication and toxication), and 8) Non-specified DAH diagnosis.

2. Definition of follow-up

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

3. Lung function

Results from available spirometry, lung volumes (total lung capacity) and diffusing capacity for carbon monoxide (DL_{CO}) were reported. Only measurements performed no later than one month after diagnosis were included for assessment of initial lung function measurement. Parameters were reported as percentage predicted. Spirometry data was used to classify lung function impairment as restrictive (FEV_1<80%, FVC<80% and FEV_1/FVC>80%) or obstructive (FEV_1<80%, and FEV_1/FVC<80%).

4. 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 (IQ range), while 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) 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 work-up and one was miscategorised. Detailed information on contributing centres is available in 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 Table 1 and Table 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 gender distribution was 57% females (ranging from 20-70%). 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=.67).

Clinical work-up

High-resolution CT (HRCT) (94%), bronchoalveolar lavage (BAL) (85%) and echocardiography (80%) were the most frequently performed diagnostic procedures (Table 3). Lung biopsy was performed in 42% and genetic testing was performed in 52 (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 of diagnostic work-up performed in each sub-group is available in supplementary data (Table S2 and Table 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 (Table S4).

Treatment

Systemic corticosteroid (CCS) was the most frequently reported medical treatment (93% of the patients), administered as IV high-dose pulse methylprednisolone (15-30 mg/kg for 3 days) (69%) or oral prednisolone (78%). Seventy-two percent of all patients received treatment CCS with both IV pulses and oral prednisolone. Monotherapy with systemic CCS was used in 43% of patients while CCS were supplemented with either hydroxychloroquine, azathioprine or both in 23%, 14% and 12% of patients, respectively (Figure 4). Other frequently administered medical treatments included Mycophenolate mofetil, Cyclosporine, Cyclophosphamide and Rituximab as the most frequent (Table S5). Two patients (a patient diagnosed with IPH and a patient with STAT3 mutation) required lung transplantation.

The median (IQR) treatment duration of IV 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), 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 TLC with an improvement of 21% and 17% ($p<0.001$), respectively. Follow-up HRCT or x-ray was performed in

101 patients (86%) with a median (IQR) time of 2.5 (1.0;7.0) years after the baseline radiology, Figure 5. 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 HRCT/chest x-ray (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. Fifteen (13%) patients died (Figure 3 and 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 gender 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 (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, 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 appeared 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) were 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). Trisomy 21 is thought to be associated with IPH although the literature is conflicting with 1/108 (0.9%) to 9/34 (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% vs. 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 under-diagnosed in the remaining patients. In 13% of patients who had BAL performed no

HLM were found, although in approximately 50% of these fresh bleeding was described. HLM is 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 (PFT) 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 (approximately 22%) from the IPH cohort into more specific sub-groups (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 - 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 a suggestion of a diagnostic approach to DAH in children and descriptive clusters to guide further research(28).

A better understanding of the pathogenesis may potentially lead to a more accurate diagnosis. A paper by Saha et al. reviews different hypothesis of IPH pathogenesis and suggest that bleeding in IPH is caused by bioactive proteins like histamine, eosinophilic cationic protein (ECP), and possibly vascular endothelial growth factor (VEGF). Further studies are needed to fully understand the pathogenesis of IPH(29).

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 used against chILD in general. The mainstay of treatment was systemic CCS (92%) independent of the underlying condition. CCS are used in other groups of chILD with no clear evidence as to whether IV high-dose pulses are more effective than oral daily administration. However, fewer side effects from CCS pulse treatment have been reported (9,23). Hydroxychloroquine and azathioprine were frequently combined with SCC and are known to have an immunomodulatory effect and especially the former 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 (30). A prospective randomised phase 2 trial of hydroxychloroquine treatment in chILD of all causes has recently been published. The study, which included 2 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 (31). 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 CCS alone or in combination with hydroxychloroquine suggesting that azathioprine is not used as first-line treatment. Combination treatment with CCS, hydroxychloroquine and azathioprine was more frequently seen in patients with IPH and auto-inflammatory disease, compared to the other patient subgroups. This may 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 (6,7,10,14,32). 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 additional significantly improved by longer or more aggressive treatment or 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 was 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 (14,33), but high compared to smaller IPH cohort studies: the French RespiRare cohort and an Indian cohort in which 1/25 (4%) and 2/26 (9%) had a fatal outcome, respectively (6,8,32). 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 (33). They found a significantly higher number of patients who tested ANA positive in the group of unfavourable patients (7 vs 1). 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 (16,34). 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 reviewed lung transplantation(35). 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 2 received lung transplantation of whom 1 was diagnosed with IPH who 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 pulmonologist, 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 include very few patients due to the rarity of the disease and for the entire study, results and statistical analyses on such small groups should always be interpreted with caution. As pulmonary function data originates from three decades obvious variations in used reference material may be expected, but most tertiary centres may have used GLI as a standard for the last decade (36). 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 (37). 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 categorization 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 all 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 and evolving into a chronic, stable condition. The variation in diagnostic workup (BAL, echocardiography, lung biopsy and laboratory work-up), 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, at term allow to determine evidence-based treatment and follow-up recommendations.

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Author's contribution

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Table 1. Diagnosis in patients with diffuse alveolar haemorrhage

Diagnosis	n
All DAH patients	**117**
Idiopathic pulmonary haemosiderosis (IPH)	**36**
DAH associated with autoimmune features	**20**
Non-specific but ANA positive	8
Non-specific ANCA associated vasculitis	9
Non-specific but SMA positive	1
Non-specific but positive rheumatoid factor	2
Systemic and collagen disorders	**18**
Granulomatosis with polyangiitis (GPA)	6
Microscopic polyangiitis	3
Polyarthritis nodosum	1
Churg-Strauss Syndrome	2
Anti-glomerular basement membrane disease	3
Systemic lupus erythematosus (SLE)	1
Antiphospholipid syndrome	1
Membranous glomerulopathy	1
Immuno-allergic disorders	**10**
Pulmonary hemorrhage with cow's milk antibody (IgG)	4
Lane-Hamilton syndrome	6
Other chILD	**5**
Pulmonary Interstitial Glycogenosis (PIG)	2
Postinfectious Bronchiolitis Obliterans (PIBO)	1
Surfactant protein C (SP-C) disorder	1
Non-specific 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
Non-specified DAH diagnosis	**4**

DAH; Diffuse alveolar haemorrhage, ANCA; Anti Neutrophil Cytoplasm Antibodies, ANA; Antinuclear Antibodies, chILD; Childhood Interstitial Lung Disease.

Table 2. Baseline characteristics and age at debut and diagnosis

Diagnosis	**n**	**Females, n (%)**	**Age at debut, years**	**Age at diagnosis, years.**	**Diagnostic delay, months**	**Trisomy 21, n (%)**
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%)
Non-specified DAH diagnosis	4	1 (25%)	12 (6.8;15,3)	10.3 (8.6;15.8)	1 (0;1)	1 (25%)

Data is presented as median (IQR) or percentage. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary haemorrhage, chILD; childhood Interstitial Lung Disease.

Table 3 Diagnostic workup in 117 patients with DAH

	N (%) who were tested	N (%) of those examined who tested positive
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 (50)	
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)	
Antineutrophilic cytoplasmic antibodies (ANCA)	100 (85)	17 (17)
Antinuclear antibodies (ANA)	96 (82)	17 (18)
Anti-double stranded DNA and anti-smooth-muscle antibodies (SMA)	74 (63)	7 (9)
Rheumatoid factor, IgM (RF)	66 (56)	10 (15)

Anti-endomysium antibodies (AEA)	51 (44)	5 (10)
Specific immunoglobulins		
Anti-transglutaminase (ATA), IgA	69 (59)	12 (17)
Anti-transglutaminase (ATA), IgG	55 (47)	4 (7)
Cow's milk, IgG	37 (32)	6 (14)

HRCT; High-resolution CT, BAL; Bronchoalveolar lavage, HLM; haemosiderin-laden macrophage, Echo; echocardiography, DPH; Diffuse pulmonary haemorrhage, IPH; Idiopathic pulmonary haemosiderosis, chILD; Childhood Interstitial Lung Disease, *NKX2.1*; NK Homeobox 1, *SFTPC*; surfactant protein C, *TBX4*; T-box transcription factor 4, *COPA*; COPI coat complex subunit alpha, *STAT-3*; Signal transducer and activator of transcription 3, *ATP7B*; ATPase cobber transporting beta; *CYBB*; cytochrome B-245 beta chain.

*Percentage not reported since the number of patients who had been tested for each condition/mutation is uncertain. [#]HLA DQA1; human leucocyte antigen DQ alpha 1, HLA DQB1 and human leucocyte antigen DQ beta 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 beta) (pulmonary alveolar proteinosis), n=1.

Figure legends

Figure 1 Inclusion process and distribution of patients with diffuse alveolar haemorrhage

DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary haemorrhage, chILD; childhood Interstitial Lung Disease.

Figure 2 symptoms and clinical presentation

The proportion of symptoms in each subgroup reported as percentages. Noteworthy, one patient may have more than one symptom. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary haemosiderosis, chILD; Childhood Interstitial Lung Disease.

Figure 3 Medical treatment

a) Percentage of patients in each sub-group treated with systemic corticosteroids (CCS), Hydroxychloroquine (HCQ), Azathioprine or none of the above. b) Percentage of patients receiving different combination of treatment in each sub-group. Only patients who have received treatment are included. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary haemosiderosis, chILD; childhood Interstitial Lung Disease.

Figure 4 Outcome

Patient outcome in the DAH cohort and the different sub-groups presented in percentage. healthy; chronic clinical stable (chronic condition with impaired lung function and/or persistent abnormal radiology but off medication for DAH); active clinical stable (under current treatment); unstable (symptomatic and/or impaired lung function despite treatment); alive but unknown condition; unknown and death.

DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary haemosiderosis, chILD; childhood interstitial lung disease.

Figure 5 Radiology and follow-up

HRCT of two patient before and after medical treatment. a) Pre-treatment HRCT of a female patient with debut of anaemia and recurrent lower respiratory infections with debut at age of 2.1 years. HRCT was performed at time of diagnosis at age of 4.8 years. b) latest HRCT performed at 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 debut of cough, dyspnoea, tachypnoea, cyanosis, haemoptysis, recurrent lower airway infection at age 6 months. HRCT was performed at time of diagnosis at the age of two years. d) latest 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.

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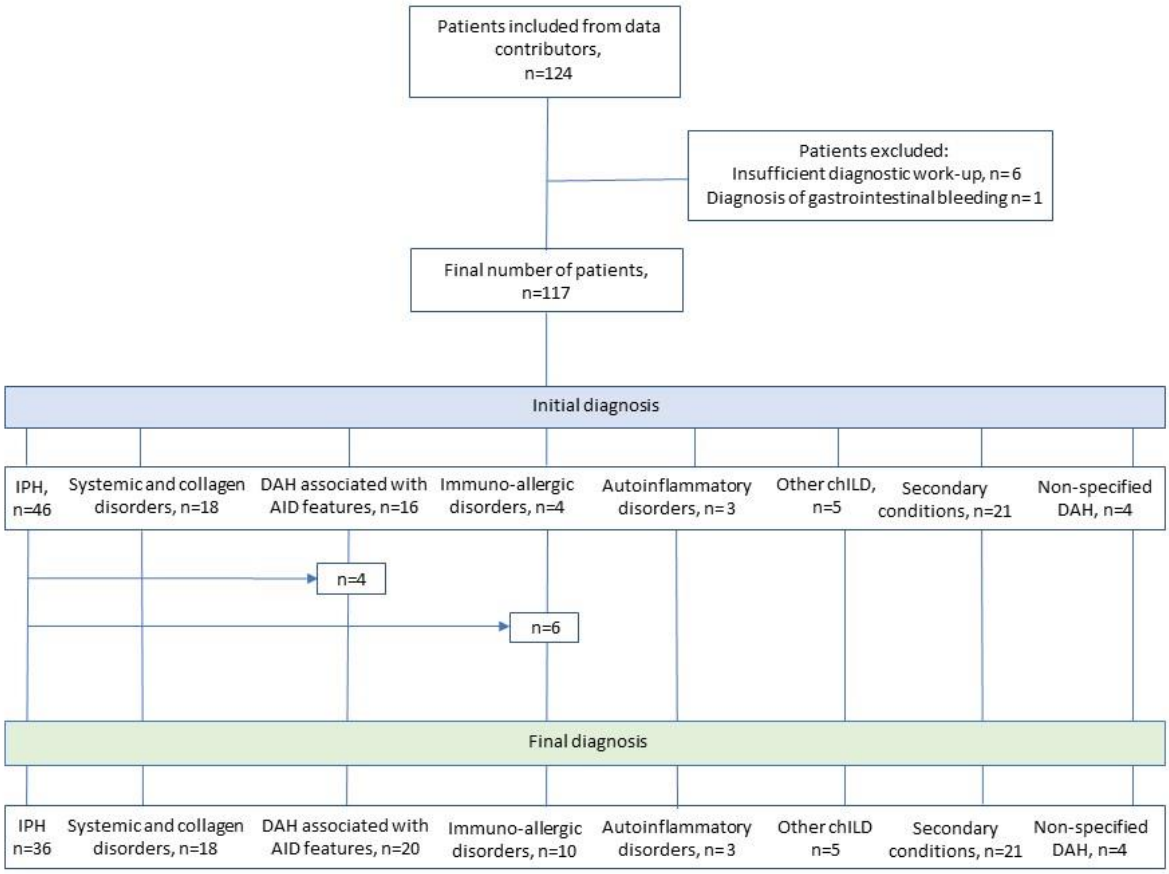
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Insufficient diagnostic work-up, n=6
Diagnosis of gastrointestinal bleeding n=1
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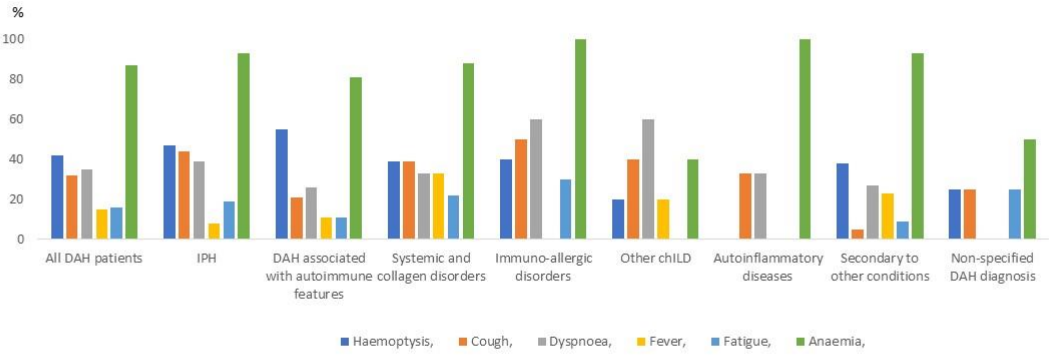
Systemic and collagen disorders, n=18
DAH associated with AID features, n=16
Immuno-allergic disorders, n=4
Autoinflammatory disorders, n=3
Other chILD, n=5
Secondary conditions, n=21
Non-specified DAH, n=4
n=4
n=6
Final diagnosis
IPH n=36
Systemic and collagen disorders, n=18
DAH associated with AID features, n=20
Immuno-allergic disorders, n=10
Autoinflammatory disorders, n=3
Other chILD n=5
Secondary conditions, n=21
Non-specified DAH, n=4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IPH
DAH associated with autoimmune features
Systemic and collagen disorders
Immuno-allergic disorders
Other chILD
Autoinflammatory diseases
Secondary to other conditions
Non-specified DAH diagnosis
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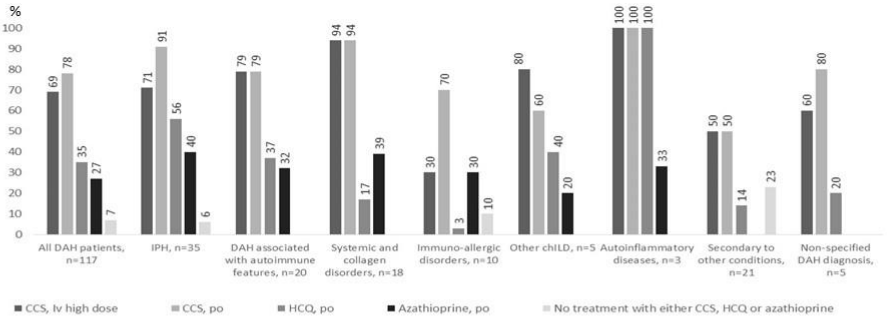
Cough,
Dyspnoea,
Fever,
Fatigue,
Anaemia,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100 100 100 33
50 50 14 23
60 80 20
All DAH patients, n=117
IPH, n=35
DAH associated with autoimmune features, n=20
Systemic and collagen disorders, n=18
Immuno-allergic disorders, n=10
Other chILD, n=5
Autoinflammatory diseases, n=3
Secondary to other conditions, n=21
Non-specified DAH diagnosis, n=5
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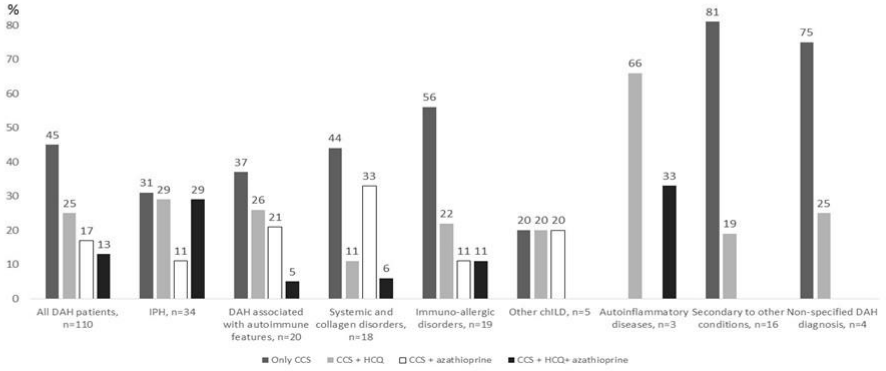
CCS, po
HCQ, po
Azathioprine, po
No treatment with either CCS, HCQ or azathioprine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DAH associated with autoimmune features, n=20
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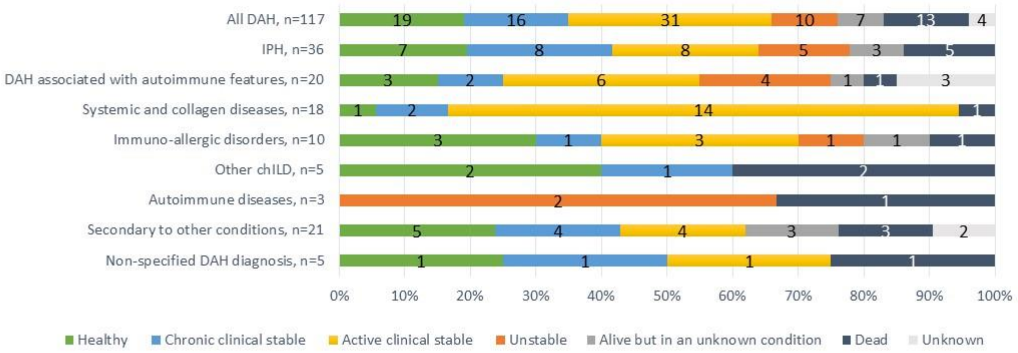
CCS + HCQ
CCS + azathioprine
CCS + HCQ+ azathioprine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Immuno-allergic disorders, n=10
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Other chILD, n=5
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Autoimmune diseases, n=3
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Secondary to other conditions, n=21
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3
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2
Non-specified DAH diagnosis, n=5
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1
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Healthy
Chronic clinical stable
Active clinical stable
Unstable
Alive but in an unknown condition
Dead
Unknown

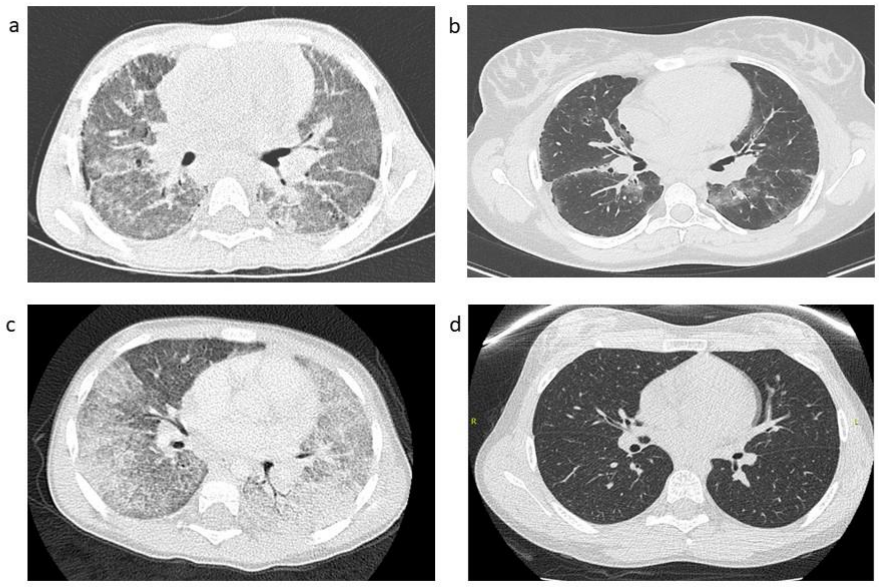
a
b
c
d

Table 1S List of data contributors

Country	City	Centre	No of cases
Belgium	Leuven	Pediatric pulmonology, KUL UZ Gasthuisberg	2
the Czech Republic	Olomouc	Dept. of Pediatrics, University Hospital Olomouc	1
Denmark	Copenhagen	Paediatric Pulmonary Service, Dept of Paediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet	5
Denmark	Aarhus	Danish Center of Pediatric Pulmonology and Allergology, Department of Pediatrics and Adolescents Medicine, University Hospital of Aarhus,	1
England	London	Royal Brompton Hospital, London, United Kingdom	2
France	Paris	APHP-Sorbonne Université, Pediatric Pulmonology and Reference Center for rare lung diseases RespiRare, Inserm U933 Laboratory of childhood genetic diseases, Armand Trousseau Hospital	13
France	Lyon	Université de Lyon, Hôpital Femme Mère Enfant, Pediatric pulmonology department	1
France	Strasbourg	Strasbourg University, Hôpital de Hautepierre, Pediatric pulmonology department	1
France	Toulouse	Dept of Pediatric Pulmonology and Allergy, Children's University Hospital,	1
Germany	Hannover	Clinic for Paediatric Pneumology, Allergology and Neonatology, Hannover Medical School, German Center for Lung Research (DZL)	21
Germany	Essen	Clinic for pediatrics III, University hospital Essen	12

Greece	Athens	2nd Pulmonary Medicine Department General University Hospital, Athens Medical School National and Kapodistrian University of Athens	2
Ireland	Dublin	CHI at Tallaght University Hospital,	1
Palestine	East Jerusalem	Pediatric department, Makassed	6
Poland	Warsaw	Department of Pediatric Pneumonology and Allergy, Medical University of Warsaw	9
Portugal	Lisbon	Department of Pediatrics, Respiratory Unit. Hospital de Santa Maria, Centro Hospitalar Lisboa Norte	2
Portugal	Porto	Departamento de Ginecologia Obstetrícia e Pediatria, Faculdade de Medicina, Universidade do Porto and Serviço de Pediatria, Centro Hospitalar Universitário de S. João	1
Spain	Barcelona	Pediatric Pulmonology and Allergology Department. Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Univestitat Autónoma de Barcelona	3
Spain	Palma de Mallorca	Pediatric pulmonology department, Son Espases University Hospital	3
Spain	Valencia	Pediatric pulmonology. Clinical Hospital of Valencia	1
Sweden	Gothenburg	Devision of Paediatrics, Drottning Silvias barn- och ungdomssjukhus, Univercity of Gothenburg	9
Turkey	Ankara	Gazi University Faculty of Medicine, Department of Pediatric Pulmonology	12
Turkey	Ankara	Hacettepe University Faculty of Medicine, Department of Pediatric Pulmonology	6
Turkey	Ankara	Department of Pediatrics, Pediatric Pulmonology Division, Ankara University Faculty of Medicine	4

Turkey	Istanbul	Division of Paediatric Pulmonology, Marmara University Faculty of Medicine	3

Data contributors are arranged by country in alphabetical order. The median (range) number of included patients per centre was 2 (1;21).

Table 2S. Detailed data of diagnostic workup in each sub-group

	HRCT		BAL			Lung biopsy				Echocardiography		Genetic analysis	
	n (%)	GGO, n (% of tested)	n (%)	HLM, n (% of tested)	Fresh bleeding, n (% of tested)	n (%)	Hemochromatosis/ HLM, n (% of tested)	Vasculitis/ capillaritis, n (% of tested)	Fibrosis, n (% of tested)	n (%)	PAH n (% of tested)	Tested, n (%)	Mutation
All DAH patients n =117	110 (94)	78 (71)	100 (85)	74 (74)	28 (28)	49 (42)	25 (51)	3 (6)	8 (16)	94 (80)	11(12)	52 (44)	
IPH n=36	34 (94)	23 (68)	29 (81)	25 (86)	9 (31)	15 (42)	10 (67)	0	3 (20)	29 (81)	2(7)	15 (42)	Trisomy 21 (n=3) Other[$] (n=1) No mutation (n=11) Pending (n=1)
DAH associated with autoimmune features n=20	17 (85)	13 (76)	18 (90)	13 (72)	6 (33)	11 (55)	4 (36)	0	2 (18)	15 (75)	0	11 (55)	NKX2.1 (n=1) No mutation (n=9) Pending (n=1)
Systemic and collagen diseases n=18	18 (100)	12 (67)	14 (78)	9 (64)	6 (43)	4 (22)	3 (75)	1 (25)	0	15 (83)	1 (7)	3 (17)	HLA DQA1*05 positive, HLA DQB1*02 positive (n=1) 22q11 del (n=1) No mutation (n=1)
Immuno-allergic diseases n=10	9 (90)	7 (78)	9 (90)	7 (78)	1 (11)	2 (20)	2 (100)	0	0	10 (100)	1 (10)	1 (10)	No mutation (n=1)
Other chILD n=5	4 (80)	3 (75)	5 (100)	4 (80)	2 (40)	5 (100)	3 (60)	1 (20)	1 (20)	4 (80)	3 (75)	4 (80)	STPTC (n=1) NKX2.1 (n=1) TBX4 (n=1) Unknown (n=1)

Autoinflammatory n=3	3 (100)	3 (100)	3 (100)	3 (100)	0	3 (100)	0	0	1(33)	2 (66)	1 (50)	3(100)	COPA (n=2) STAT-3 (n=1)
Secondary to other conditions n=21	22 (88)	18 (82)	20 (80)	13 (65)	4 (20)	9 (36)	2 (22)	1 (11)	1 (11)	18 (72)	3 (17)	13 (62)	Trisomy 21 (n=2) ATP7B (n=1) PFIC 1 (n=1) WAS (n=1) FLT3-ITD (n=1) WT1 (n=1) No mutation (n=6)
Non-specified DAH diagnosis =5	3 (75)	2 (67)	2 (50)	NA	NA	0	0	0	0	3 (75)	0	2 (50)	CYBB (n=1) Trisomy 21 (n=1) No mutation (n=1)

HRCT; High-resolution CT, BAL; Bronchoalveolar lavage, Echo; echocardiography, DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, chILD; Childhood Interstitial Lung Disease, HLA DQA1; human leucocyte antigen DQ alpha 1, HLA DQB1; human leucocyte antigen DQ beta 1, 22q11; DeGeorges syndrome, NKX2.1; NK Homeobox 1, STPTC; surfactant protein C, TBX4; T-box transcription factor 4, COPA; COPI coat complex subunit alpha, STAT-3; Signal transducer and activator of transcription 3, ATP7B; ATPase cobber transporting beta; PFIC-1; progressive familial intrahepatic cholestasis 1, WASp; Wiscott -Aldrich Syndrome, FLT3-ITD; fms-like tyrosine kinase 3 internal tandem duplication, WT1; Wilms' tumor 1; CYBB; cytochrome B-245 beta chain. [$]Heterozygote for CSF2RB (colony-stimulating factor 2 receptor subunit beta) (pulmonary alveolar proteinosis).

Table 3S Autoantibodies and specific immunoglobulins

	ATA, IgA	ATA, IgG	antigliadin antibodies (AGA)	ANCA	ANA	Anti-endomysium antibodies (AEA)	SMA	RF	Cow's milk, IgG
All DAH patients, n=117	12/69	4/55	9/56	17/100	17/96	5/51	7/74	10/66	5/37
IPH, n=36	0/22	1/16	1/20	0/28	0/28	0/18	0/18	0/21	0/14
DAH associated with autoimmune features, n=20	0/13	0/10	0/9	7/18	8/18	0/8	4/15	6/13	1/6
Systemic and collagen diseases, n=18	2/8	0/6	0/5	10/18	4/16	1/7	1/12	1/7	0/4
Immuno-allergic diseases, n=12	7/9	3/9	6/8	0/9	1/10	4/8	0/8	1/9	4/6
Other chILD condition, n=5	1/1	0/1	0/2	0/4	0/4	0/1	0/2	0/2	0/1
Autoinflammatory diseases, n=3	0/3	0/3	1/3	0/3	2/3	0/1	1/3	1/3	0/1
Secondary to other conditions, n=21	2/11	0/9	1/7	0/17	2/15	0/6	1/14	1/9	0/5
Non-specified DAH diagnosis, n=4	0/2	0/1	0/2	0/3	0/2	0/2	0/2	0/2	0

Results are presented as number tested positive / number tested. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, chILD; Childhood Interstitial Lung Disease, ATA; anti transglutaminase antibody, ANCA; antineutrophilic cytoplasmic antibodies, ANA; antinuclear antibodies, SMA; anti-double-stranded DNA and anti-smooth-muscle antibodies; RF; rheumatoid factor.

Table 4S. Results from first available pulmonary function test.

	FEV1, % pred.	FVC, % pred.	FEV1/FVC, % pred.	TLC, % pred.	DLCO, % pred.	KCO, % pred.
All DAH patients, n=117	66 (56;86) n=36	68 (56;86) n=36	96 (84;106) n=36	87 (72;106) n= 34	69 (58;91) n= 26	77 (67;92) n=10
IPH, n=36	74 (53;79) n=9	77 (70;81) n=9	97 (94;104) n=9	73 (65;91) n=5	61 (54;69) n=7	75 (69;80) n=2
DAH associated with autoimmune features, n=20	61 (59;61) n=5	59 (50;67) n=5	97 (90;102) n=5	91 (68;99) n=6	54 (43;83) n=4	67 n=1
Systemic and collagen diseases, n=18	78 (66;85) n=11	78 (60;86) n=11	97 (84;106) n=11	89 (79;104) n=10	75 (68;94) n=9	77 (71;85) n=3
Immuno-allergic diseases, n=12	86 (44;98) n=3	90 (66;98) n= 3	91 (66;106) n=3	106 (105;106) n=2	94 (90;97) n=2	87 (76;98) n=2
Other chILD condition, n=5	51 (45;57) n=2	59 (50;68) n=2	65 n=2	114 n=1	69 n=1	NA
Autoinflammatory diseases, n=3	27 n=1	24 n=1	88 n=1	73 n=1	72 n=1	NA
Secondary to other conditions, n=21	71 (63;95) n= 6	66 (60;88) n=6	103 (93;107) n=6	81 (77;85) n= 6	100 (55;113) n=3	91 (83;98) n=2
Non-specified DAH diagnosis, n=4	NA	NA	NA	NA	NA	NA

Data is presented as median (IQ-range) unless n <5 in which case median (range) is presented. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, chILD; Childhood Interstitial Lung Disease, FEV1; Forced Expiratory Volume in the first second, FVC; Forced Vital Capacity, TLC; Total Lung Capacity, DLCO; the diffusion capacity for Carbon Monoxide, KCO; Carbon monoxide transfer coefficient n = the number of patients in which the data was available. NA; no data available.

Table 5S. Most frequent medical treatments besides corticosteroids, hydroxychloroquine, and azathioprine

	Non-specific immuno-suppressive drugs	Immuno-globulins	cytostatic	Biological treatment	plasmapheresis
All DAH patients, n=117	16 (14%)	3 (3%)	11 (9%)	11 (9%)	6 (5%)
IPH, n=36	3 (8%) Mycophenolate mofetil, 2 Cyclosporine, 1	0	4 (11%) 6-mercaptopurine, 2 Cyclophosphamide, 2	1 (3%) Rituximab, 1	0
DAH associated with autoimmune featuresn=20	3 (15%) Mycophenolate mofetil, 3	1 (5%)	2 (10%) Cyclophosphamide, 2	2 (10%) Rituximab, 1 Baricitinib, 1	0
Systemic and collagen diseases, n=18	8 (44%) Mycophenolate mofetil, 7 Cyclosporine, 3		5 (28%) Methotrexate, 2 Cyclophosphamide, 3	5 (28%) Rituximab, 4 Mepolizumab, 1	6 (33%)
Immuno-allergic diseases, n=10	0	0	0	0	0
Other ILD condition, n=5	0	1 (20%)	0	1 (20%) Tocilizumab	0
Autoinflammatory diseases, n=3	0	0	0	2 (33%) Baricitinib, 2 Canakinumab, 1 Rituximab, 1	0
Secondary to other conditions, n=21	2 (10%) Mycophenolate mofetil, 2	1 (5%)	0	0	0
Non-specified DAH diagnosis, n=4	0	0	0	0	0

DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, chILD; Childhood Interstitial Lung Disease.

Table 6S. Follow-up data: time of follow-up, chest imagining, and pulmonary function.

	Time to follow-up		Follow-up HRCT or chest x-ray	Follow-up pulmonary function					
	from debut, yrs	from diagnosis, yrs	follow-up HRCT or x-ray with abnormalities Assoc. with DAH	FEV1, % pred.	FVC, % pred.	FEV1/FVC, % pred.	TLC, % pred.	DLCO, % pred.	KCO, % pred.
All DAH patients, n=117	4 (1.5;7.8)	3.2 (1.2;7.0)	60% (61/101)	84 (66;97) n=20	87 (69;96) n=63	96 (86;101) n=63	100 (85;112) n=42	87 (66;99) n=47	91 (83;94) n=21
IPH, n=36	8.8 (5.2;16.0)	7.4 (4.8;14.6)	62% (18/29)	74 (66;93) n=17	79 (68;87) n=17	91 (86;97) n=17	99 (95;112) n=9	83 (70;93) n=14	87 (73;99) n=7
DAH associated with autoimmune features, n=20	4.4 (3.1;7.0)	3.9 (1.7;9.4)	68% (13/19)	61 (60;73) n=11	70 (60;78) n=11	86 (82;98) n=11	85 (74;94) n=9	71 (53;83) n=8	89 n=2
Systemic and collagen disorders, n=18	3.7 (1.8;5.5)	3.4 (1.7;5.0)	56% (9/16)	99 (94;112) n=16	101 (95;116) n=16	97 (95;99) n=16	108 (103;120) n=11	100 (85;113) n=14	98 (86;122) n=4
Immuno-allergic disorders, n=10	5.0 (2.6;7.0)	3.8 (1.3;6.3)	55% (5/9)	87 (71;103) n=8	88 (86;98) n= 8	85 (77;100) n=8	114 (105;129) n=4	92 (60;120) n=4	88 (66;114) n=4
Other chILD, n=5	1.5 (0.9;6.9)	1.5 (0.9;7.3)	60% (3/5)	83 (30;95) n=3	92 (30;93) n=3	107 (88;108) n=3	124 (108;140) n=2	93 (82;103) n=2	78 n=1
Autoimmune disorders, n=3	13.0 (12.8;17.8)	11.0 (5.8;14.9)	100% (2/2)	44 (23;64) n=2	42 (21;62) n=2	100 (61;106) n=2	58 (39;76) n=2	NA	NA
Secondary to other conditions, n=21	2.4 (1.8;3.0)	2.3 (1.7;3.4)	44% (8/18)	93 (81;96) n= 11	87 (77;95) n=11	101 (93;108) n=11	90 (81;100) n= 4	87 (55;97) n=2	91 (74;95) n=3
Non-specified DAH diagnosis, n=4	4.5 (0.7;32.4)	4.4 (0.6;32.4)	50% (2/4)	72 n=1	69 n=1	104 n=1	90 n=1	NA	NA

Data are presented as median (IQ-range) unless other is specified. DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, chILD; Childhood Interstitial Lung Disease, FEV1; Forced Expiratory Volume in the first second, FVC; Forced Vital Capacity, TLC; Total Lung Capacity, DLCO; the diffusion capacity for Carbon Monoxide, VA; Alveolar Volume. n = the number of patients in which the data was available, yrs; years.

Table 7S. Description of patients who have passed away

Diagnoses	Age at debut, years	Gender	Cause of death	Age at time of death, years	Period from debut to time of death	Medical treatment	
						Type of medication	Months of treatment
IPH	7.0 years	Female	Acute bleeding	7.4 years	4.0 months	Methylprednisolone, iv Prednisolone, po Azathioprine, po	NA NA NA
IPH	7.5 years	Female	Acute bleeding	9 years	18 months	Prednisolone, po	9 (8030 mg)
IPH	2.8 years	Female	Acute bleeding	4.9 years	1.9 years	Prednisolone, po	12 (8800 mg)
IPH	0.3	Female	Acute rejection after lung transplantation	NA	NA	Methylprednisolone, iv Prednisolone, po Hydroxychloroquine, po Cyclosporine	NA NA NA NA
IPH	9.3	Female	Chronic respiratory failure	13.3 years	4.0 years	Methylprednisolone, iv Prednisolone, po Hydroxychloroquine, po Azathioprine, po Cyclophosphamide	NA 46 39 6 NA
Anti-glomerular basement membrane disease	12.8	Female	Chronic respiratory failure	12.9 years	1 month	Methylprednisolone, iv Hydroxychloroquine, po Cyclophosphamide	1 155 NA
STAT-3 mutation	2.0	Male	Died after lung transplantation	14.8 years	12.8 years	Methylprednisolone, iv Prednisolone, po Hydroxychloroquine, po Etanercept Mycophenolate mofetil	NA NA NA NA NA
Pulmonary interstitial glycogenosis (PIG)	0.0	Male	Multiorgan failure	0.05	0.5 months	Methylprednisolone, iv	1
Lung fibrosis	5.8 years	Male	Chronic respiratory failure	14.9 years	9.1 years	Methylprednisolone, iv Prednisolone, po Immunoglobulins Azathioprine, po Tocilizumab	NA 109 NA 3 NA

Pulmonary hemorrhage with cow's milk antibody (IgG)	8 months	Female	Acute bleeding	11 months	3 months	Other	
Cantu-syndrome	9 months	Male	Pulmonary infection	3.3 years	2.4 years	methylprednisolone, iv Prednisolone, po Other	28 months NA
DAH associated with autoimmune features (ANCA positive)	15.2 years	Male	Pulmonary aspergillosis	16.7 years	18 months	methylprednisolone, iv Cyclophosphamide	1 NA
DAH developed secondary to infection	9 months	Male	Pulmonary infection	2.5 years	22 months	methylprednisolone, iv	1
Bone marrow transplant-related lung injury	14.3 years	Female	DAH/idiopathic pneumonia syndrome after allogeneic bone marrow transplantation	15.6 years	16 months	Other	NA
non-specified DAH diagnosis	17 years	Male	Pulmonary aspergillosis	17.4 years	3.6 months	NA	NA

DAH; Diffuse alveolar haemorrhage, IPH; Idiopathic pulmonary Hemosiderosis, ANCA; Anti-neutrophil cytoplasm antibodies.