Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral immunodeficiencies

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Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral immunodeficiencies

--Manuscript Draft--

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Abstract:	Clinical Implications: Interstitial Lung Disease (ILD) in children often indicates life-threatening PID and has a poor prognosis without hematopoietic stem cell transplantation. Lung biopsy often provides definitive pathological results that help to direct management decisions.

INPRACTICE-D-19-00055, Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral immunodeficiencies

POINT BY POINT RESPONSE

COMMENTS FROM REVIEWER #1:

In this case series, the authors present the details of 9 pediatric patients with PIDD and ILD, screened from a cohort of 1034 pediatric referrals to their PIDD clinic. This adds to the literature since little has been published on this topic to date specific to the pediatric population.

Comment: Page 4, Line 61 - Suggest to highlight here the important observation that in this cohort, in all but two cases, the age of onset of chest symptoms preceded the diagnosis of lung disease, which in turn preceded the diagnosis of PIDD.

Response: As suggested, a sentence highlighting this important point as been added to the text.

Page 4 Line 78 - Would highlight that the lack of observed granulomata is in definite contrast to the granulomatous inflammation seen in GLILD associated with CVID.

Response: The referee raises a good point. Contrary to what might be assumed from the descriptive term, GLILD is not usually associated with granuloma, but rather more diffuse collections of histiocytes and lymphocytes. This point has now been highlighted and referenced in the text.

Comment: Page 5 Last sentence — There appears to be a missing word "HSCT can be life-saving but should be carried out by experienced transplant physicians as post-transplant clinical course, as severe inflammation of the lungs, liver and gut are common in this group of patients."

Response: The sentence has now been rephrased.

Table I - the IgG levels are relatively high in most cases, please clarify if this is reactive or if patients were on supplemental immunoglobulin?

Response: None of the patients were on immunoglobulins replacement, those with high IgG were reactive. The fact that some patients have high reactive IgG are detailed in the Supplementary case histories.

Comment: Supplement Page 14 Line 26 - typo in word "splice"

Response: Typo corrected.

COMMENTS FROM REVIEWER #2:

This is important and helpful information in an unusual complication of a rare disease. It is first to describe interstitial lung diseases in relation to phenotype in children such detail so it is just a pity that it is a short communication but even so - it will be useful. The cases are particularly helpful and interesting - this should be encouraged in this journal when PIDs (which are rare) are discussed.

I have a few suggestions

Comment: * CVID was designated Common Variable Immunodeficiency Disorders by the IUIS in 2009 - as this is a diagnosis of exclusion

Response: The full expanded name of the disorder has now been added.

* GLILD -this term is confusing and most clinical immunologists are trying to avoid using it

Response: The authors fully agree with the reviewer's comment, and have kept the use of term GLILD to a minimum in this manuscript – using it only two in the manuscript.

Comment: * It is not clear what roles of the non-Manchester authors played; presumably in diagnosis of each case but this should be stated.

Response: Professor Hambleton, although based in Newcastle upon Tyne, does regular joint pediatric immunology outpatient clinics in Manchester to provide advice and input on our complex patients – her affiliation with the Department of Paediatric Allergy and Immunology in Manchester has now detailed in the author affiliation list. Colleagues (Latour, Picard and Rieux-Laucat) from the Imagine Institute in Paris provided important input, determining the definitive genetic diagnosis of a number of the patients in this report. This information has now been added to the acknowledgement section.

Comment: * Given that CVIDs in adults are mentioned early on, it would be helpful to compare the findings with those reported in these adults - some CVID patients have combined immune deficiencies, much like children.

Response: Some of the histological features (particularly the lack of well-formed granuloma) of GLILD in adults with CVID have now been compared with the histological results in this cohort (see response to second point by referee 1).

Comment: * A stronger statement about survival in relation to HSCT would be helpful too

Response: As recommended, the statement about survival and outcome in relation to HSCT has been strengthened (second last line of text). It has also been highlighted in the “Clinical implications” paragraph after the beginning of the text.

Comment: * Were the follicles described in patients 2 and 4 B cell follicles with GC or was there no immunochemistry?

Response: In both patient 2 and 4, there were some B-cell follicles which stained with CD20, as shown in Figure 1 (CGD and LRBA respectively).

COMMENTS FROM THE EDITORIAL OFFICE:

Comment: ** Please remove the list of key words from the manuscript.

Response: As requested, key word list has now been removed.

Comment: ** Please remove the closure (listing of authors at the end of the manuscript text body).

Response: List of authors at end of manuscript removed as requested.

Comment: ** Replace the Highlights Box with the required Clinical Implications statement: 1-2 sentences (maximum 40 words) that summarize the clinical implications and importance of the report.

Response: Highlights Box replaced with Clinical Implications statement as requested.

Comment: ** On the title page, please include a conflict of interest statement disclosing all financial and organizational relationships for ALL authors of the manuscript. Authors should disclose all potential conflicts of interest including funding sources that supported their work and any commercial and/or organizational associations. These include consultant arrangements, speakers' bureau participation, stock or other equity ownership, patent licensing arrangements, support such as financial or materials grants for research, employment, or expert witness testimony. If an author has no conflicts to disclose, this should be included in the statement. Further information can be found at <http://www.elsevier.com/conflictsofinterest> and at http://service.elsevier.com/app/answers/detail/a_id/286/supporthub/publishing. This statement should be viewed and approved by all authors.

Response: A conflict of interest statement has now been included on the front page of the manuscript.

Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral immunodeficiencies

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There were no external sources of funding. None of the authors declare any conflict of interests in relation to this study.

~~Key words: primary immunodeficiency disease, children, interstitial lung disease, GLILD HSCT~~

25 **Clinical Implications:** Interstitial Lung Disease (ILD) in children often indicates life-
26 threatening PID and has a poor prognosis without hematopoietic stem cell transplantation.

~~1. What is already known about this topic? Interstitial Lung Disease (ILD) occurs in 20% of adults with COVID, usually of unclear genetic etiology. In children, there are only a few reports linking ILD to specific primary immunodeficiency diseases (PID).~~

27 Lung biopsy often provides definitive pathological results that help to direct management
28 decisions.

To the Editor,

Recurrent acute and chronic lung infections associated with bronchiectasis are a major cause of morbidity and mortality in patients with Primary Immunodeficiency Disorders (PID).¹⁻³ In adults, inflammatory Interstitial Lung Disease (ILD) not due to pyogenic infection is found in up to 20% of patients with Common Variable Immunodeficiency Disorders (CVID) and can be associated with progressive restrictive lung disease and shortened survival.⁴⁻⁵ High resolution chest CT scan is the gold standard imaging technique for diagnosing ILD. Where there is doubt about the diagnosis lung biopsy is recommended.^{1,2} Histology classically shows lymphocytic infiltrates and/or granuloma, which in adults with CVID is termed Granulomatous-Lymphocytic Interstitial Lung Disease (GLILD).⁶ Although there are reports of ILD associated with a number of monogenic PIDs including Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA4), Lipopolysaccharide (LPS)-Responsive and Beige-like Anchor protein (LRBA), Recombination-Activating Gene (RAG1) deficiencies, X-linked Inhibitor of Apoptosis Protein (XIAP) and Chronic Granulomatous Disease (CGD),^{2,7,8} there are no published series delineating the prevalence, clinical characteristics, histologic features and management of children with PID and ILD. This retrospective case series aimed to review the genotype and clinicopathological phenotype of children presenting to our tertiary pediatric primary immunodeficiency center at Royal Manchester Children's Hospital, Manchester, United Kingdom between 2005 and 2018 with ILD and PID who underwent detailed investigations including chest CT scans and in most cases lung biopsy. We hypothesized that ILD in children often indicates life-threatening primary immunodeficiency disease and has a poor prognosis without hematopoietic stem cell transplantation (HSCT).

The case note review was part of a Natural History and Causes of CVID study approved by the Local Research Ethics Committee (03/ST/016). The demography, immunology, histology and genetic screening of nine patients (0.9%) with PID and ILD out

of 1,034 new pediatric referrals to our service because of recurrent, unusual or serious infections or inflammatory diseases were collated (Table I, Figure 1, and online supplementary material). This series of patients includes all but one patient known to Departments of Paediatric Histopathology, Immunology and Respiratory Medicine who had lung biopsies which demonstrated ILD associated with lymphocytic infiltration and/or granuloma formation.

Seven of the nine patients were males. Seven were Asian. All presented with a chronic cough and breathlessness suggestive of recurrent chest infections or asthma. In all but one case in which there was a family history of PID, it was the onset of chest symptoms that prompted a search for an underlying immunodeficiency disorder. A genetic diagnosis was confirmed in seven patients. Three children had a primary neutrophil immunodeficiency (two p40phox Chronic Granulomatous Disease (CGD) and one G6PC3 deficiency), and four a primary T-cell immunodeficiency (*RAG1*, *STK4*, *LRBA*, *ITK*). Whole exome sequencing of an eighth patient with pulmonary EBV-driven LPD uncovered a potentially pathogenic variant in a gene involved in T-cell signalling and further experiments to confirm the functional relevance of this novel finding are in progress. The ninth patient died from pulmonary Hemophagocytosis LymphoHistiocytosis (HLH) in 2006. Limited candidate gene screening at the time was negative and there was insufficient DNA available for subsequent whole exome sequencing. The median age of onset of chest symptoms of the nine patients was 12 months old. Except for one male who presented in the second year of life with *RAG1* deficient Severe Combined Immunodeficiency (SCID) and died of progressive multifocal leukoencephalopathy, and a teenage boy who died of fulminant pulmonary HLH, the remaining seven patients were alive 12 months to 14 years post-HSCT.

Lung biopsies assisted to direct clinical management (Figure 1). Tuberculosis and fungal infections were excluded, allowing for the discontinuation of anti-microbials. No

evidence of vasculitis was found in any of the patient's biopsies. Although granulomata were only seen in the patients with CGD, a number of the patients had a prominent histiocytic (CD68) infiltrate. This is in keeping with the lung histology of GLILD in adults with CVID, which is characterized by loose, more diffuse collections of histiocytic and lymphocytic infiltrates rather than well-defined clusters of histiocytes and multinuclear giant cells typical of sarcoidosis.⁵ In the three patients with primary neutrophil immunodeficiencies and the patient with the LRBA deficiency, lung biopsy confirmed the diagnosis of an inflammatory IBD rather than pyogenic infection, and responded to high dose corticosteroids prior to HSCT. In the four patients with primary T-cell immunodeficiencies, lung biopsies confirmed EBV-driven LPD, allowing focused treatment with rituximab ± EBV-directed cytotoxic T-cells or chemotherapy. The lung biopsies of the teenager who died showed a mixed histiocytic, T- and B-lymphocytic infiltrate and evidence of hemophagocytosis. Infection, including EBV-driven LPD and vasculitis were excluded.

In conclusion, this is the first case series to delineate the variation in genotype and the frequent life-threatening clinical phenotype of children with ILD complicating PID. Lung biopsy often provides important information that helps to direct management decisions, not only by ruling out fungal and mycobacterial infections and vasculitis, but also in tailoring therapy based on the presence of inflammatory infiltrates or EBV-driven LPD. Diagnosis of the underlying PID is challenging, as patients may not only present with classical phenotypes such as EBV-positive ILD secondary to ITK deficiency, but also with unusual manifestations, for example "late-onset" SCID, or p40phox CGD where the standard dihydrorhodamine test may be normal.^{8,9} HSCT can be life-saving- and should be considered as a definitive, curative therapeutic option. It is however important that HSCT is ~~but should be~~ carried out by experienced transplant physicians, as the post-transplant clinical course can be complicated by, ~~as~~ severe inflammation of the lungs, liver and gut ~~are common~~ in this group of patients.

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153 **Figure Legends**

154

155 **Figure 1 Characteristic lung biopsies of different PID associated with interstitial lung**
156 **disease.** Panels (top to bottom) show Hematoxylin & Eosin (H&E), CD3 (T-cells), CD20 (B-
157 cells), CD68 (histiocytes), EBV *in situ* hybridization (EBV) for each condition.

158 **Table I Patient characteristics**

patient number	gene defect	Dx ^a	age of onset of chest disease (m)	age at lung disease Dx (m)	age at PID Dx (m)	gender	race ^b	age HSCt (m)	outcome	hemoglobin (g/l)	platelets (x 10 ⁹ /l)	lymphocytes (x10 ⁹ /l)	IgM (g/l)	IgG (g/l)	IgA (g/l)	CD4 (x 10 ⁶ /l)	CD8 (x 10 ⁶ /l)	CD19 (x 10 ⁶ /l)	CD56 (x 10 ⁶ /l)	lung histology
1	<i>NCF4</i>	CGD	24	31	36	f	A	56	alive	119	215	3.04	1.0	12	2.1	1058	933	1415	129	G
2	<i>NCF4</i>	CGD	8	44	56	f	A	62	alive	125	297	1.88	0.98	13.8	1.76	906	766	3755	32	L
3	<i>G6PC3</i>	G6PC3	1	97	112	m	A	142	alive	128	58	0.49	0.44	17.9	0.32	339	271	164	23	L
4	<i>LRBA</i>	LRBA	3	138	138	m	A	144	alive	80	173	1.8	1.27	7.65	0.48	495	282	794	39	L
5	<i>ITK</i>	CID	7	10	1	m	A	12	alive	124	351	3.11	1.32	6.6	0.87	2072	978	993	162	E-L*
6	<i>STK4</i>	CID	12	12	142	f	A	144	alive	105	348	1.30	1.06	31.1	4.89	100	570	270	200	E-L
7	<i>RAG1</i>	CID	14	22	27	m	O	-	died	111	510	0.17	1.56	6.82	<0.1	19	50	37	221	E-L
8	?	CID	48	69	-	m	A	98	alive	138	340	3.8	3	21	30	800	1887	283	69	Φ
9	?	HLH	168	180	-	m	C	-	died	97	60	0.41	5.02	11.2	1.22	260	200	60	20	L

160 Dx: diagnosis, PID: primary immunodeficiency disease, *NCF4*: Neutrophil Cytosolic Factor 4, *ITK*: IL-2 Inducible T-Cell Kinase, *STK4*:
161 Serine/Threonine Kinase 4, *RAG1*: Recombination Activating Gene 1, ^aCGD: chronic granulomatous disease, G6PC3: Glucose-6-Phosphatase
162 Catalytic Subunit 3 deficiency, LRBA: lipopolysaccharide (LPS)-responsive and beige-like anchor protein deficiency, CID: combined T/B-cell
163 immunodeficiency, ^bC: Caucasian A: Asian, O: Other. Lung histology: non-caseating granuloma (G), lymphoid hyperplasia (L), lymphoma (L*),
164 EBV (E), Φ - EBV-lymphoproliferative disease diagnosed after finding EBV from sputum, nodular shadowing on CT scan images and response
165 to rituximab.

1 **Online material**

2 **Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral**
3 **immunodeficiencies**

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13 Newcastle, Newcastle upon Tyne, United Kingdom

14 **Clinical case vignettes** (high resolution chest CT scans are shown in Figure S1)

15 **Patient 1** A four-year-old Pakistani girl presented at the age of two years old with a chronic
16 cough and clubbing, associated with cervical lymphadenopathy and hepatosplenomegaly.
17 Parents were first cousins. There was no family history of chest problems or tuberculosis. A
18 chest x-ray showed bilateral, multinodular shadowing. Chest CT scan confirmed patchy
19 infiltrates in the right and left lower lobes, but no bronchiectasis. Hilar lymphadenopathy was
20 noted, particularly on the right (7 x 10mm). Bronchioalveolar lavage revealed no microbes.
21 Lymph node biopsy showed granulomatous inflammation without caseation. Stains for
22 mycobacteria and fungi were negative. Because of the chronic unremitting nature of the lung
23 disease a lung biopsy was performed, which showed granulomatous lymphocytic infiltration
24 with no organisms identified.

25 Standard dihydrorhodamine test with PMA was normal, but stimulation with *E. coli* was
26 reduced. Gene sequencing revealed a homozygous c.118-1G>A splice variant in the *NCF4* gene
27 consistent with p40phox CGD.⁸ Parents were both heterozygous carriers. The symptoms did not
28 respond to anti-microbials and could only partly be controlled with intermittent pulse
29 intravenous methylprednisolone.

30 In view of the diagnosis of CGD and her ongoing inflammatory lung disease, she
31 underwent matched-sibling HSCT at the age of three years old. Post-transplant course was
32 complicated by mild veno-occlusive disease treated with defibrotide, followed by fulminant
33 inflammatory disease of her gut, liver and lungs, complicated by pulmonary hemorrhage,
34 requiring high frequency oscillatory ventilation in intensive care. The lung disease was brought
35 under control with anti-TNF therapy and extra-corporal photopheresis. Twelve months post-
36 transplant she developed autoimmune thrombocytopenia, which responded to rituximab.

37 Eighteen months post-transplant, her condition is stable and systemic immune suppression has
38 been discontinued. Peripheral leukocytes are 100% donor.

39 **Patient 2** A seven-year-old Pakistani girl presented with a history of chronic chest symptoms
40 going back to when she was eight months old. She had a chest deformity, clubbing and oxygen
41 saturations in air of 88%. Her growth and development were delayed and she had intermittent
42 fevers and an iron deficiency anemia. Parents were first cousins. No pathogens had been isolated
43 from her chest. Because of wheeze and breathlessness, she had been treated with recurrent
44 courses of oral prednisolone. Chest x-ray and CT scan showed hilar lymphadenopathy and
45 bilateral patchy shadowing and nodules especially in both lower lobes. There was no
46 bronchiectasis. Lung biopsy showed interstitial inflammation with lymphoid follicle formation.
47 There was alveolar remodelling, prominent type 2 pneumocyte hyperplasia and abundant intra-
48 alveolar proteinaceous exudates.

49 Dihydrorhodamine test with PMA was normal, but stimulation with *E. coli* was reduced.
50 Gene sequencing revealed a homozygous c.32+2T>G variant in *NCF4* consistent with p40phox
51 CGD. Both parents were found to be carriers. Her inflammatory lung disease was only partly
52 controlled with pulse methylprednisolone therapy. She therefore underwent matched unrelated
53 HSCT at the age of five years old. Three months post-transplantation, she developed lung EBV
54 LPD, which was treated with rituximab. Four months post-transplant she developed a central
55 line infection complicated by *Klebsiella pneumoniae* septicemia, cardiac tamponade and
56 cardiogenic shock requiring intensive care. Two years post transplantation she is now well and
57 off all medication.

58 **Patient 3** A 12-year-old Pakistani boy suffered from episodes of cough, wheeze and
59 breathlessness from one month of age. He had been treated with multiple courses of antibiotics,
60 inhaled steroids, nebulized bronchodilators and oral prednisolone. He also had chronically loose

61 stools. His parents were first cousins. He was referred to our primary immunodeficiency centre
62 at the age of nine years old after moving to the UK. He had been diagnosed with a congenital
63 neutropenia at the age of six years and started on regular G-CSF, although continued to have
64 persistent cough and dyspnea, with poor exercise tolerance requiring supplementary night time
65 oxygen. FEV₁ and FVC were 30% of predicted. Stool culture, bronchoscopy and
66 bronchoalveolar lavage revealed no pathogens. Mantoux and Quantiferon tests were negative.

67 High resolution chest CT scan showed air trapping and nodular opacities in the left lower
68 lobe, with only mild basal bronchiectasis. Lung biopsy showed peri-bronchial mononuclear
69 infiltrate with occasional lymphoid nodules with no granuloma, fungi or mycobacteria. There
70 was no fibrosis or pulmonary hypertensive changes. Gut biopsies showed inflammation with
71 cryptitis, crypt abscesses, inflammatory infiltrate throughout the colon but no granuloma. There
72 were also patchy inflammatory changes in the esophagus, stomach and terminal ileum.

73 In view of his congenital neutropenia, symmetrical growth retardation, cryptorchidism
74 and distended veins on his anterior chest, genetic testing was performed which confirmed a
75 homozygous frameshift c882_903 duplication in the *G6PC3* gene. Because of frequent hospital
76 admissions for his lung disease and poorly controlled inflammatory bowel disease he underwent
77 matched unrelated HSCT. Fifteen months post-transplant, he is now able to scooter along the flat
78 and not needing supplementary oxygen. His stool frequency has also decreased. Post-transplant
79 immune suppression has been discontinued with 100% donor engraftment.

80 **Patient 4** An eleven-year-old Indian boy presented in infancy with chronic watery diarrhea due
81 to an autoimmune non-granulomatous colitis. He was treated for four years with corticosteroids,
82 to which azathioprine was added. His parents were first cousins. At seven years old he
83 developed generalized atopic dermatitis and at eight years old cervical lymphadenopathy,
84 splenomegaly, chronic urticaria, a Coombs positive hemolytic anemia and ITP with epistaxis,

85 which only partly responded to corticosteroids, methotrexate and IV rituximab. At the age of ten
86 years old, while on the methotrexate (10mg/m²/week), he developed ILD (EBV positive by PCR
87 on sputum but not blood) with fevers and signs of respiratory distress. High resolution chest CT
88 scan showed confluent dense consolidation in both lower lobes, multiple areas of hazy nodular,
89 ground glass changes and hilar lymphadenopathy. Lung biopsy showed a diffuse, reactive
90 lymphoid hyperplasia with lymphoid follicle formation. At the same time his autoimmune
91 cytopenias became more difficult to control. His lung disease and cytopenias only partly
92 responded to rituximab, and required high dose systemic corticosteroids.

93 Whole exome sequencing revealed a homozygous c.5770_5776 deletion in the *LRBA*
94 gene. Both parents were carriers. As there were no suitable matched sibling or unrelated donors,
95 he underwent a maternal haploidentical peripheral blood TCRαβ–depleted HSCT with reduced
96 intensity conditioning. His post-transplant course was uncomplicated. Four years post-
97 transplant he off all medication and has 100% donor engraftment. His chest disease, autoimmune
98 enteropathy and cytopenias have resolved. Because of proteinuria, he investigated and diagnosed
99 with diffuse mesangial sclerosis, which is currently not progressive and he has normal renal
100 function and no proteinuria.

101 **Patient 5** Prompted by a family history, a 15-year-old boy diagnosed with a homozygous
102 c.626G>A *ITK* deficiency at the age of one month old. HSCT was also delayed because of
103 Hirschsprung's disease requiring surgery in the neonatal period and the difficulty finding a
104 suitable donor. At the age of seven months, EBV viremia was noted and chest CT scan showed
105 widespread bulky masses throughout both lungs. The largest of these replaced most of the
106 right upper lobe. There were multiple smaller nodules scattered throughout both lungs. Lung
107 biopsy showed replacement of normal parenchyma with an EBV-driven diffuse large B-cell
108 lymphoma. He was treated with rituximab and UK CCSG NHL901 chemotherapy protocol,

109 followed by a single allelic A mismatched related donor transplant. He remains well off all
110 therapy 14 years post HSCT.

111 **Patient 6** A 16-year-old Asian girl presented at the age of three years old with a history of
112 recurrent infections from 14 months old, when she developed viral croup requiring ventilation
113 and cryptosporidial diarrhea. Over the next few years, she suffered from recurrent *Streptococcus*
114 *pneumoniae* pneumonia and ear infections, as well as recurrent thrush, molluscum contagiosum
115 and herpes gingivostomatitis requiring intravenous acyclovir. She had a chronic productive
116 cough, which did not respond to asthma inhalers. Examination revealed tachypnea and oxygen
117 saturations in air of 94%, a discharging left ear and oral thrush.

118 Investigations showed a CD4 lymphopenia but raised, reactive serum immunoglobulins
119 and protective vaccine antibody responses. HIV screen was negative. EBV was detected in the
120 blood by PCR. Chest CT scan showed multiple small nodules of different sizes throughout both
121 lungs and no bronchiectasis. Lung biopsy showed a B-cell infiltrate positive for EBV. Her lung
122 EBV-lymphoproliferative disease was treated with rituximab. Parents were first cousins and two
123 other sisters also had a CD4 lymphopenia associated with opportunistic infections, both dying of
124 infectious complications post-HSCT.

125 The patient was initially treated conservatively with prophylactic valaciclovir, co-
126 trimoxazole, fluconazole. However, at the age of 12 years old she developed an extra-nodal B-
127 cell lymphoma within her left submaxillary salivary gland with evidence of paraprotein bands in
128 her blood. Whole exome sequencing confirming a *STK4* c.442C>T gene variant in the patient
129 and both her affected siblings. Parents were heterozygous carriers. Because of the progressive
130 disease, the patient underwent matched-sibling HSCT. Post-transplant course was complicated
131 by CMV reactivation with diarrhea, which responded to ganciclovir. The patient is currently
132 well four years post-HSCT.

133 **Patient 7** A Turkish Cypriot boy presented at the age of 20 months old with a history of
134 recurrent cough, wheeze and progressive dyspnea, which started at 14 months old. He had been
135 admitted to hospital five times, once to the pediatric intensive care with “asthma”, which only
136 partly responded to inhaled bronchodilators and short courses of oral prednisolone. There was
137 no consanguinity or significant family history.

138 Blood tests showed persistent lymphopenia ($0.5 \times 10^9/L$) with $CD3^{low}CD19^{low}CD56^{normal}$
139 lymphocyte subsets. Serum IgA was undetectable but IgM and IgG were normal, as were
140 vaccine responses to tetanus, *Haemophilus influenzae* type B, measles and 4/12 pneumococcal
141 serotypes. Chest CT scan showed extensive nodules in both lungs, particularly in the lower
142 lobes. Respiratory secretions at bronchoalveolar lavage were positive for EBV by PCR. Lung
143 biopsy showed a nodular, T-cell rich lymphohistiocytic infiltrate with EBV positivity
144 demonstrated by *in situ* hybridization. A diagnosis of lung EBV-driven lymphoproliferative
145 disease was made and the patient was treated with rituximab and EBV-directed cytotoxic T-
146 lymphocyte infusions. Concurrently, the patient developed a rapidly progressive encephalopathy
147 with hemiplegia and brain stem dysfunction requiring intensive care. Brain MR scan was
148 indicative of progressive multifocal encephalopathy. Blood and urine BK, JC and astrovirus
149 PCR were all negative. The patient deteriorated and died at the age of 21 months old. Post-
150 mortem was refused. Genetic results received after death showed a homozygous *RAG1*
151 c.2333G>A variant. Both parents were heterozygous carriers.

152 **Patient 8** A Pakistani boy presented at the age of four years old with an eight-week history of
153 fevers and weight loss and hepatomegaly. Chest x-ray showed extensive consolidation and chest
154 CT scan showed diffuse interstitial changes with fine reticulation and small nodular opacities in
155 all lobes, particularly the right upper lobe, middle lobes and left lower lobes. There was no
156 bronchiectasis. He was initially treated for tuberculosis, although there was no evidence of TB
157 on culture, Mantoux or Quantiferon testing. EBV was identified by PCR from his sputum (log

158 4.5) and blood (log 4.1), although EBV IgM and IgG were both negative. Lymphocyte subsets
159 were normal and serum immunoglobulins were all raised. Whole exome sequencing was
160 performed and no variants in known genes caused PID was revealed, but a potentially
161 pathogenic mutation in a novel gene has been identified and functional tests are ongoing.

162 The EBV lymphoproliferative disease was successfully treated with rituximab, and thus
163 although lung biopsy had been considered to rule out lymphoma it was not performed. He
164 underwent a matched unrelated HSCT and remains well and asymptomatic two years post-
165 transplantation.

166 **Patient 9** A previously well white European male presented in 2005 at the age of 14 years old
167 with night sweats, vomiting, anorexia and weight loss but no diarrhea. There was no
168 consanguinity or significant family history. An episode of chickenpox at three years old was no
169 more severe than normal. Endoscopy showed active gastritis (*Helicobacter pylori* negative) with
170 no evidence of inflammatory bowel disease. At 15 years old, following viral respiratory illness
171 (which other family members also suffered), he developed a cough, progressive breathlessness,
172 bilateral pleural effusions and respiratory failure. Laboratory investigations showed a
173 generalized lymphopenia ($0.53 \times 10^9/L$), thrombocytopenia and hyperferritinemia (5,636 $\mu g/L$),
174 but normal mitogen responses, serum immunoglobulin concentrations, ACE and neutrophil
175 oxidative burst test.

176 Chest CT scan showed extensive reticulonodular interstitial shadowing, particularly in
177 both lower lobes, with relative sparing of the upper lobes. Lung biopsy demonstrated an intra-
178 alveolar histiocytic and T-cell infiltrate with follicle formation. There were no granulomata.
179 EBV, fungi and acid-fast bacilli were absent.

180 His lung disease did not respond to pulse methylprednisolone or methotrexate. He was ventilated
181 in intensive care, but died one week later from progressive lung failure. Post-mortem histology

182 of the lungs showed extensive infiltration of macrophages and lymphocytes, with diffuse
183 alveolar damage and hemorrhage. There was no evidence of infection, vasculitis or granuloma.
184 No EBV or CMV, or acid-fast bacilli were seen. Gut tissue showed no evidence of inflammatory
185 bowel disease. Bone (rib), lung, liver and lymph nodes all show prominent histiocytes with
186 hemophagocytosis. Candidate ADA/PNP, XAP and FHLH gene screen was normal, and
187 unfortunately there was insufficient DNA available to conduct whole exome sequencing after the
188 patient's death.

189 **Figure legends**

190 **Figure S1 Chest high resolution CT scans**

191 See clinical case vignettes for description of pathology.

Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral immunodeficiencies

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22 **Clinical Implications:** Interstitial Lung Disease (ILD) in children often indicates life-
23 threatening PID and has a poor prognosis without hematopoietic stem cell transplantation.
24 Lung biopsy often provides definitive pathological results that help to direct management
25 decisions.

To the Editor,

Recurrent acute and chronic lung infections associated with bronchiectasis are a major cause of morbidity and mortality in patients with Primary Immunodeficiency Disorders (PID).¹⁻³ In adults, inflammatory Interstitial Lung Disease (ILD) not due to pyogenic infection is found in up to 20% of patients with Common Variable Immunodeficiency Disorders (CVID) and can be associated with progressive restrictive lung disease and shortened survival.⁴⁻⁵ High resolution chest CT scan is the gold standard imaging technique for diagnosing ILD. Where there is doubt about the diagnosis lung biopsy is recommended.^{1,2} Histology classically shows lymphocytic infiltrates and/or granuloma, which in adults with CVID is termed Granulomatous-Lymphocytic Interstitial Lung Disease (GLILD).⁶ Although there are reports of ILD associated with a number of monogenic PIDs including Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA4), Lipopolysaccharide (LPS)-Responsive and Beige-like Anchor protein (LRBA), Recombination-Activating Gene (RAG1) deficiencies, X-linked Inhibitor of Apoptosis Protein (XIAP) and Chronic Granulomatous Disease (CGD),^{2,7,8} there are no published series delineating the prevalence, clinical characteristics, histologic features and management of children with PID and ILD. This retrospective case series aimed to review the genotype and clinicopathological phenotype of children presenting to our tertiary pediatric primary immunodeficiency center at Royal Manchester Children's Hospital, Manchester, United Kingdom between 2005 and 2018 with ILD and PID who underwent detailed investigations including chest CT scans and in most cases lung biopsy. We hypothesized that ILD in children often indicates life-threatening primary immunodeficiency disease and has a poor prognosis without hematopoietic stem cell transplantation (HSCT).

The case note review was part of a Natural History and Causes of CVID study approved by the Local Research Ethics Committee (03/ST/016). The demography, immunology, histology and genetic screening of nine patients (0.9%) with PID and ILD out

of 1,034 new pediatric referrals to our service because of recurrent, unusual or serious infections or inflammatory diseases were collated (Table I, Figure 1, and online supplementary material). This series of patients includes all but one patient known to Departments of Paediatric Histopathology, Immunology and Respiratory Medicine who had lung biopsies which demonstrated ILD associated with lymphocytic infiltration and/or granuloma formation.

Seven of the nine patients were males. Seven were Asian. All presented with a chronic cough and breathlessness suggestive of recurrent chest infections or asthma. In all but one case in which there was a family history of PID, it was the onset of chest symptoms that prompted a search for an underlying immunodeficiency disorder. A genetic diagnosis was confirmed in seven patients. Three children had a primary neutrophil immunodeficiency (two p40phox Chronic Granulomatous Disease (CGD) and one G6PC3 deficiency), and four a primary T-cell immunodeficiency (*RAG1*, *STK4*, *LRBA*, *ITK*). Whole exome sequencing of an eighth patient with pulmonary EBV-driven LPD uncovered a potentially pathogenic variant in a gene involved in T-cell signalling and further experiments to confirm the functional relevance of this novel finding are in progress. The ninth patient died from pulmonary Hemophagocytosis LymphoHistiocytosis (HLH) in 2006. Limited candidate gene screening at the time was negative and there was insufficient DNA available for subsequent whole exome sequencing. The median age of onset of chest symptoms of the nine patients was 12 months old. Except for one male who presented in the second year of life with *RAG1* deficient Severe Combined Immunodeficiency (SCID) and died of progressive multifocal leukoencephalopathy, and a teenage boy who died of fulminant pulmonary HLH, the remaining seven patients were alive 12 months to 14 years post-HSCT.

Lung biopsies assisted to direct clinical management (Figure 1). Tuberculosis and fungal infections were excluded, allowing for the discontinuation of anti-microbials. No

evidence of vasculitis was found in any of the patient's biopsies. Although granulomata were only seen in the patients with CGD, a number of the patients had a prominent histiocytic (CD68) infiltrate. This is in keeping with the lung histology of GLILD in adults with CVID, which is characterized by loose, more diffuse collections of histiocytic and lymphocytic infiltrates rather than well-defined clusters of histiocytes and multinuclear giant cells typical of sarcoidosis.⁵ In the three patients with primary neutrophil immunodeficiencies and the patient with the LRBA deficiency, lung biopsy confirmed the diagnosis of an inflammatory IBD rather than pyogenic infection, and responded to high dose corticosteroids prior to HSCT. In the four patients with primary T-cell immunodeficiencies, lung biopsies confirmed EBV-driven LPD, allowing focused treatment with rituximab $\pm$ EBV-directed cytotoxic T-cells or chemotherapy. The lung biopsies of the teenager who died showed a mixed histiocytic, T- and B-lymphocytic infiltrate and evidence of hemophagocytosis. Infection, including EBV-driven LPD and vasculitis were excluded.

In conclusion, this is the first case series to delineate the variation in genotype and the frequent life-threatening clinical phenotype of children with ILD complicating PID. Lung biopsy often provides important information that helps to direct management decisions, not only by ruling out fungal and mycobacterial infections and vasculitis, but also in tailoring therapy based on the presence of inflammatory infiltrates or EBV-driven LPD. Diagnosis of the underlying PID is challenging, as patients may not only present with classical phenotypes such as EBV-positive ILD secondary to ITK deficiency, but also with unusual manifestations, for example "late-onset" SCID, or p40phox CGD where the standard dihydrorhodamine test may be normal.^{8,9} HSCT can be life-saving and should be considered as a definitive, curative therapeutic option. It is however important that HSCT is carried out by experienced transplant physicians, as the post-transplant course can be complicated by severe inflammation of the lungs, liver and gut in this group of patients.

101

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106 for a number of the complex patients described in this report and helped draft the manuscript.

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134 **Figure Legends**

135

136 **Figure 1 Characteristic lung biopsies of different PID associated with interstitial lung**
137 **disease.** Panels (top to bottom) show Hematoxylin & Eosin (H&E), CD3 (T-cells), CD20 (B-
138 cells), CD68 (histiocytes), EBV *in situ* hybridization (EBV) for each condition.

139 **Table I Patient characteristics**

patient number	gene defect	Dx ^a	age of onset of chest disease (m)	age at lung disease Dx (m)	age at PID Dx (m)	gender	race ^b	age HSCt (m)	outcome	hemoglobin (g/l)	platelets (x 10 ⁹ /l)	lymphocytes (x10 ⁹ /l)	IgM (g/l)	IgG (g/l)	IgA (g/l)	CD4 (x 10 ⁶ /l)	CD8 (x 10 ⁶ /l)	CD19 (x 10 ⁶ /l)	CD56 (x 10 ⁶ /l)	lung histology
1	<i>NCF4</i>	CGD	24	31	36	f	A	56	alive	119	215	3.04	1.0	12	2.1	1058	933	1415	129	G
2	<i>NCF4</i>	CGD	8	44	56	f	A	62	alive	125	297	1.88	0.98	13.8	1.76	906	766	3755	32	L
3	<i>G6PC3</i>	G6PC3	1	97	112	m	A	142	alive	128	58	0.49	0.44	17.9	0.32	339	271	164	23	L
4	<i>LRBA</i>	LRBA	3	138	138	m	A	144	alive	80	173	1.8	1.27	7.65	0.48	495	282	794	39	L
5	<i>ITK</i>	CID	7	10	1	m	A	12	alive	124	351	3.11	1.32	6.6	0.87	2072	978	993	162	E-L*
6	<i>STK4</i>	CID	12	12	142	f	A	144	alive	105	348	1.30	1.06	31.1	4.89	100	570	270	200	E-L
7	<i>RAG1</i>	CID	14	22	27	m	O	-	died	111	510	0.17	1.56	6.82	<0.1	19	50	37	221	E-L
8	?	CID	48	69	-	m	A	98	alive	138	340	3.8	3	21	30	800	1887	283	69	Φ
9	?	HLH	168	180	-	m	C	-	died	97	60	0.41	5.02	11.2	1.22	260	200	60	20	L

141 Dx: diagnosis, PID: primary immunodeficiency disease, *NCF4*: Neutrophil Cytosolic Factor 4, *ITK*: IL-2 Inducible T-Cell Kinase, *STK4*:
142 Serine/Threonine Kinase 4, *RAG1*: Recombination Activating Gene 1, ^aCGD: chronic granulomatous disease, G6PC3: Glucose-6-Phosphatase
143 Catalytic Subunit 3 deficiency, LRBA: lipopolysaccharide (LPS)-responsive and beige-like anchor protein deficiency, CID: combined T/B-cell
144 immunodeficiency, ^bC: Caucasian A: Asian, O: Other. Lung histology: non-caseating granuloma (G), lymphoid hyperplasia (L), lymphoma (L*),
145 EBV (E), Φ - EBV-lymphoproliferative disease diagnosed after finding EBV from sputum, nodular shadowing on CT scan images and response
146 to rituximab.

1 **Online material**

2 **Life-threatening pulmonary interstitial lung disease complicating pediatric non-humoral**
3 **immunodeficiencies**

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13 Newcastle, Newcastle upon Tyne, United Kingdom

14 **Clinical case vignettes** (high resolution chest CT scans are shown in Figure S1)

15 **Patient 1** A four-year-old Pakistani girl presented at the age of two years old with a chronic
16 cough and clubbing, associated with cervical lymphadenopathy and hepatosplenomegaly.
17 Parents were first cousins. There was no family history of chest problems or tuberculosis. A
18 chest x-ray showed bilateral, multinodular shadowing. Chest CT scan confirmed patchy
19 infiltrates in the right and left lower lobes, but no bronchiectasis. Hilar lymphadenopathy was
20 noted, particularly on the right (7 x 10mm). Bronchioalveolar lavage revealed no microbes.
21 Lymph node biopsy showed granulomatous inflammation without caseation. Stains for
22 mycobacteria and fungi were negative. Because of the chronic unremitting nature of the lung
23 disease a lung biopsy was performed, which showed granulomatous lymphocytic infiltration
24 with no organisms identified.

25 Standard dihydrorhodamine test with PMA was normal, but stimulation with *E. coli* was
26 reduced. Gene sequencing revealed a homozygous c.118-1G>A splice variant in the *NCF4* gene
27 consistent with p40phox CGD.⁸ Parents were both heterozygous carriers. The symptoms did not
28 respond to anti-microbials and could only partly be controlled with intermittent pulse
29 intravenous methylprednisolone.

30 In view of the diagnosis of CGD and her ongoing inflammatory lung disease, she
31 underwent matched-sibling HSCT at the age of three years old. Post-transplant course was
32 complicated by mild veno-occlusive disease treated with defibrotide, followed by fulminant
33 inflammatory disease of her gut, liver and lungs, complicated by pulmonary hemorrhage,
34 requiring high frequency oscillatory ventilation in intensive care. The lung disease was brought
35 under control with anti-TNF therapy and extra-corporal photopheresis. Twelve months post-
36 transplant she developed autoimmune thrombocytopenia, which responded to rituximab.

37 Eighteen months post-transplant, her condition is stable and systemic immune suppression has
38 been discontinued. Peripheral leukocytes are 100% donor.

39 **Patient 2** A seven-year-old Pakistani girl presented with a history of chronic chest symptoms
40 going back to when she was eight months old. She had a chest deformity, clubbing and oxygen
41 saturations in air of 88%. Her growth and development were delayed and she had intermittent
42 fevers and an iron deficiency anemia. Parents were first cousins. No pathogens had been isolated
43 from her chest. Because of wheeze and breathlessness, she had been treated with recurrent
44 courses of oral prednisolone. Chest x-ray and CT scan showed hilar lymphadenopathy and
45 bilateral patchy shadowing and nodules especially in both lower lobes. There was no
46 bronchiectasis. Lung biopsy showed interstitial inflammation with lymphoid follicle formation.
47 There was alveolar remodelling, prominent type 2 pneumocyte hyperplasia and abundant intra-
48 alveolar proteinaceous exudates.

49 Dihydrorhodamine test with PMA was normal, but stimulation with *E. coli* was reduced.
50 Gene sequencing revealed a homozygous c.32+2T>G variant in *NCF4* consistent with p40phox
51 CGD. Both parents were found to be carriers. Her inflammatory lung disease was only partly
52 controlled with pulse methylprednisolone therapy. She therefore underwent matched unrelated
53 HSCT at the age of five years old. Three months post-transplantation, she developed lung EBV
54 LPD, which was treated with rituximab. Four months post-transplant she developed a central
55 line infection complicated by *Klebsiella pneumoniae* septicemia, cardiac tamponade and
56 cardiogenic shock requiring intensive care. Two years post transplantation she is now well and
57 off all medication.

58 **Patient 3** A 12-year-old Pakistani boy suffered from episodes of cough, wheeze and
59 breathlessness from one month of age. He had been treated with multiple courses of antibiotics,
60 inhaled steroids, nebulized bronchodilators and oral prednisolone. He also had chronically loose

61 stools. His parents were first cousins. He was referred to our primary immunodeficiency centre
62 at the age of nine years old after moving to the UK. He had been diagnosed with a congenital
63 neutropenia at the age of six years and started on regular G-CSF, although continued to have
64 persistent cough and dyspnea, with poor exercise tolerance requiring supplementary night time
65 oxygen. FEV₁ and FVC were 30% of predicted. Stool culture, bronchoscopy and
66 bronchoalveolar lavage revealed no pathogens. Mantoux and Quantiferon tests were negative.

67 High resolution chest CT scan showed air trapping and nodular opacities in the left lower
68 lobe, with only mild basal bronchiectasis. Lung biopsy showed peri-bronchial mononuclear
69 infiltrate with occasional lymphoid nodules with no granuloma, fungi or mycobacteria. There
70 was no fibrosis or pulmonary hypertensive changes. Gut biopsies showed inflammation with
71 cryptitis, crypt abscesses, inflammatory infiltrate throughout the colon but no granuloma. There
72 were also patchy inflammatory changes in the esophagus, stomach and terminal ileum.

73 In view of his congenital neutropenia, symmetrical growth retardation, cryptorchidism
74 and distended veins on his anterior chest, genetic testing was performed which confirmed a
75 homozygous frameshift c882_903 duplication in the *G6PC3* gene. Because of frequent hospital
76 admissions for his lung disease and poorly controlled inflammatory bowel disease he underwent
77 matched unrelated HSCT. Fifteen months post-transplant, he is now able to scooter along the flat
78 and not needing supplementary oxygen. His stool frequency has also decreased. Post-transplant
79 immune suppression has been discontinued with 100% donor engraftment.

80 **Patient 4** An eleven-year-old Indian boy presented in infancy with chronic watery diarrhea due
81 to an autoimmune non-granulomatous colitis. He was treated for four years with corticosteroids,
82 to which azathioprine was added. His parents were first cousins. At seven years old he
83 developed generalized atopic dermatitis and at eight years old cervical lymphadenopathy,
84 splenomegaly, chronic urticaria, a Coombs positive hemolytic anemia and ITP with epistaxis,

85 which only partly responded to corticosteroids, methotrexate and IV rituximab. At the age of ten
86 years old, while on the methotrexate (10mg/m²/week), he developed ILD (EBV positive by PCR
87 on sputum but not blood) with fevers and signs of respiratory distress. High resolution chest CT
88 scan showed confluent dense consolidation in both lower lobes, multiple areas of hazy nodular,
89 ground glass changes and hilar lymphadenopathy. Lung biopsy showed a diffuse, reactive
90 lymphoid hyperplasia with lymphoid follicle formation. At the same time his autoimmune
91 cytopenias became more difficult to control. His lung disease and cytopenias only partly
92 responded to rituximab, and required high dose systemic corticosteroids.

93 Whole exome sequencing revealed a homozygous c.5770_5776 deletion in the *LRBA*
94 gene. Both parents were carriers. As there were no suitable matched sibling or unrelated donors,
95 he underwent a maternal haploidentical peripheral blood TCRαβ–depleted HSCT with reduced
96 intensity conditioning. His post-transplant course was uncomplicated. Four years post-
97 transplant he off all medication and has 100% donor engraftment. His chest disease, autoimmune
98 enteropathy and cytopenias have resolved. Because of proteinuria, he investigated and diagnosed
99 with diffuse mesangial sclerosis, which is currently not progressive and he has normal renal
100 function and no proteinuria.

101 **Patient 5** Prompted by a family history, a 15-year-old boy diagnosed with a homozygous
102 c.626G>A *ITK* deficiency at the age of one month old. HSCT was also delayed because of
103 Hirschsprung's disease requiring surgery in the neonatal period and the difficulty finding a
104 suitable donor. At the age of seven months, EBV viremia was noted and chest CT scan showed
105 widespread bulky masses throughout both lungs. The largest of these replaced most of the
106 right upper lobe. There were multiple smaller nodules scattered throughout both lungs. Lung
107 biopsy showed replacement of normal parenchyma with an EBV-driven diffuse large B-cell
108 lymphoma. He was treated with rituximab and UK CCSG NHL901 chemotherapy protocol,

109 followed by a single allelic A mismatched related donor transplant. He remains well off all
110 therapy 14 years post HSCT.

111 **Patient 6** A 16-year-old Asian girl presented at the age of three years old with a history of
112 recurrent infections from 14 months old, when she developed viral croup requiring ventilation
113 and cryptosporidial diarrhea. Over the next few years, she suffered from recurrent *Streptococcus*
114 *pneumoniae* pneumonia and ear infections, as well as recurrent thrush, molluscum contagiosum
115 and herpes gingivostomatitis requiring intravenous acyclovir. She had a chronic productive
116 cough, which did not respond to asthma inhalers. Examination revealed tachypnea and oxygen
117 saturations in air of 94%, a discharging left ear and oral thrush.

118 Investigations showed a CD4 lymphopenia but raised, reactive serum immunoglobulins
119 and protective vaccine antibody responses. HIV screen was negative. EBV was detected in the
120 blood by PCR. Chest CT scan showed multiple small nodules of different sizes throughout both
121 lungs and no bronchiectasis. Lung biopsy showed a B-cell infiltrate positive for EBV. Her lung
122 EBV-lymphoproliferative disease was treated with rituximab. Parents were first cousins and two
123 other sisters also had a CD4 lymphopenia associated with opportunistic infections, both dying of
124 infectious complications post-HSCT.

125 The patient was initially treated conservatively with prophylactic valaciclovir, co-
126 trimoxazole, fluconazole. However, at the age of 12 years old she developed an extra-nodal B-
127 cell lymphoma within her left submaxillary salivary gland with evidence of paraprotein bands in
128 her blood. Whole exome sequencing confirming a *STK4* c.442C>T gene variant in the patient
129 and both her affected siblings. Parents were heterozygous carriers. Because of the progressive
130 disease, the patient underwent matched-sibling HSCT. Post-transplant course was complicated
131 by CMV reactivation with diarrhea, which responded to ganciclovir. The patient is currently
132 well four years post-HCST.

133 **Patient 7** A Turkish Cypriot boy presented at the age of 20 months old with a history of
134 recurrent cough, wheeze and progressive dyspnea, which started at 14 months old. He had been
135 admitted to hospital five times, once to the pediatric intensive care with “asthma”, which only
136 partly responded to inhaled bronchodilators and short courses of oral prednisolone. There was
137 no consanguinity or significant family history.

138 Blood tests showed persistent lymphopenia ($0.5 \times 10^9/L$) with CD3^{low}CD19^{low}CD56^{normal}
139 lymphocyte subsets. Serum IgA was undetectable but IgM and IgG were normal, as were
140 vaccine responses to tetanus, *Haemophilus influenzae* type B, measles and 4/12 pneumococcal
141 serotypes. Chest CT scan showed extensive nodules in both lungs, particularly in the lower
142 lobes. Respiratory secretions at bronchoalveolar lavage were positive for EBV by PCR. Lung
143 biopsy showed a nodular, T-cell rich lymphohistiocytic infiltrate with EBV positivity
144 demonstrated by *in situ* hybridization. A diagnosis of lung EBV-driven lymphoproliferative
145 disease was made and the patient was treated with rituximab and EBV-directed cytotoxic T-
146 lymphocyte infusions. Concurrently, the patient developed a rapidly progressive encephalopathy
147 with hemiplegia and brain stem dysfunction requiring intensive care. Brain MR scan was
148 indicative of progressive multifocal encephalopathy. Blood and urine BK, JC and astrovirus
149 PCR were all negative. The patient deteriorated and died at the age of 21 months old. Post-
150 mortem was refused. Genetic results received after death showed a homozygous *RAG1*
151 c.2333G>A variant. Both parents were heterozygous carriers.

152 **Patient 8** A Pakistani boy presented at the age of four years old with an eight-week history of
153 fevers and weight loss and hepatomegaly. Chest x-ray showed extensive consolidation and chest
154 CT scan showed diffuse interstitial changes with fine reticulation and small nodular opacities in
155 all lobes, particularly the right upper lobe, middle lobes and left lower lobes. There was no
156 bronchiectasis. He was initially treated for tuberculosis, although there was no evidence of TB
157 on culture, Mantoux or Quantiferon testing. EBV was identified by PCR from his sputum (log

158 4.5) and blood (log 4.1), although EBV IgM and IgG were both negative. Lymphocyte subsets
159 were normal and serum immunoglobulins were all raised. Whole exome sequencing was
160 performed and no variants in known genes caused PID was revealed, but a potentially
161 pathogenic mutation in a novel gene has been identified and functional tests are ongoing.

162 The EBV lymphoproliferative disease was successfully treated with rituximab, and thus
163 although lung biopsy had been considered to rule out lymphoma it was not performed. He
164 underwent a matched unrelated HSCT and remains well and asymptomatic two years post-
165 transplantation.

166 **Patient 9** A previously well white European male presented in 2005 at the age of 14 years old
167 with night sweats, vomiting, anorexia and weight loss but no diarrhea. There was no
168 consanguinity or significant family history. An episode of chickenpox at three years old was no
169 more severe than normal. Endoscopy showed active gastritis (*Helicobacter pylori* negative) with
170 no evidence of inflammatory bowel disease. At 15 years old, following viral respiratory illness
171 (which other family members also suffered), he developed a cough, progressive breathlessness,
172 bilateral pleural effusions and respiratory failure. Laboratory investigations showed a
173 generalized lymphopenia ($0.53 \times 10^9/L$), thrombocytopenia and hyperferritinemia (5,636 $\mu g/L$),
174 but normal mitogen responses, serum immunoglobulin concentrations, ACE and neutrophil
175 oxidative burst test.

176 Chest CT scan showed extensive reticulonodular interstitial shadowing, particularly in
177 both lower lobes, with relative sparing of the upper lobes. Lung biopsy demonstrated an intra-
178 alveolar histiocytic and T-cell infiltrate with follicle formation. There were no granulomata.
179 EBV, fungi and acid-fast bacilli were absent.

180 His lung disease did not respond to pulse methylprednisolone or methotrexate. He was ventilated
181 in intensive care, but died one week later from progressive lung failure. Post-mortem histology

182 of the lungs showed extensive infiltration of macrophages and lymphocytes, with diffuse
183 alveolar damage and hemorrhage. There was no evidence of infection, vasculitis or granuloma.
184 No EBV or CMV, or acid-fast bacilli were seen. Gut tissue showed no evidence of inflammatory
185 bowel disease. Bone (rib), lung, liver and lymph nodes all show prominent histiocytes with
186 hemophagocytosis. Candidate ADA/PNP, XAP and FHLH gene screen was normal, and
187 unfortunately there was insufficient DNA available to conduct whole exome sequencing after the
188 patient's death.

189 **Figure legends**

190 **Figure S1 Chest high resolution CT scans**

191 See clinical case vignettes for description of pathology.

Figure No. 1 - Unmarked