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REVIEW

Biomarkers to predict and diagnose pulmonary complications in children post haematopoietic stem cell transplant

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Abstract

Objectives. Haematopoietic cell transplant (HCT) is a cellular therapy for a group of high-risk children with cancer, immunodeficiency and metabolic disorders. Whilst curative for a child's underlying condition, HCT has significant risks associated, including lung injury. These complications are associated with increased post HCT mortality and require improved methods of risk stratification, diagnosis and treatment. Methods. Biomarkers measured in bronchoalveolar fluid and peripheral blood have been identified for both acute and chronic lung injury post HCT.This review evaluates the current research available investigating the use of these biomarkers to improve clinical care, with a focus on the paediatric cohort. Results. Elevated levels of cytokines such as IL-6, IL-8, G-CSF and TNF were identified as potential predictive biomarkers for the development of post HCT lung disease. The pulmonary microbiome was found to have strong potential as a biomarker pre and post HCT for the development of pulmonary complications. General limitations of the studies identified were study design, retrospective or single centre and not exclusively performed in the paediatric population. Conclusion. To translate biomarker discovery into clinical implementation further research is required, utilising larger cohorts of children in prospective trials to validate these biomarkers and determine how they can be translated into better outcomes for children post HCT.

Keywords: haematopoietic stem cell transplant, paediatric, pulmonary

INTRODUCTION

Pulmonary complications after haematopoietic stem cell transplant

Haematopoietic cell transplant (HCT) is a cellular therapy used in children with a range of haematological conditions, immunodeficiency syndromes and metabolic disorders. Whilst HCT offers a cure for the child's underlying condition, it has a range of infectious and inflammatory complications that can occur, including pulmonary complications.[1] Pulmonary complications occur in approximately 25–60% of children following HCT and contribute to 25–65% of non-relapse mortality.[2,3]

Pulmonary complications are frequently classified as infectious or non-infectious, with the latter divided into acute or chronic lung injury determined by onset post HCT. Acute lung injury, classically occurring in the first 100 days post HCT, includes idiopathic pneumonia syndrome (IPS), defined[4] as widespread lung injury demonstrated on imaging and by respiratory symptoms, in the absence of a lower respiratory tract infection. Diffuse alveolar haemorrhage (DAH), thought to be a subset of IPS with predominant vascular endothelial dysfunction, also occurs in the early stages post HCT and portends a worse prognosis. Chronic lung injury post HCT classically occurs later than 100 days post HCT and includes diagnoses of bronchiolitis obliterans syndrome (BOS),[1,2] restrictive lung disease (RLD) and cryptogenic organising pneumonia. Due to advances in supportive care and treatment with broad-spectrum antimicrobials, outcomes from infectious pulmonary complications have improved in recent years.[5] In contrast, with more limited methods for early detection and targeted therapy, the prognosis for patients with non-infectious pulmonary complications remain poor.

The categorisation of infective versus non-infective pulmonary complications is often too simple to encapsulate the lung disease that occurs in children post HCT; alternatively, pulmonary complications are often the result of a series of cumulative insults. These insults arise from pre HCT treatments, disruptions to the pulmonary microbiome, infectious pathogens, endothelial dysfunction, dysregulated inflammation and graft factors particularly mismatched donor source[5–11]. As a result, multi-modal therapy, including antimicrobials, immune modulating agents and ventilatory support in the intensive care unit (ICU) is often required. Current methods to detect and diagnose pulmonary complications include direct sampling of the cells and organisms within the lung bronchoalveolar fluid, commonly via bronchoalveolar lavage (BAL). BAL fluid is considered representative of the cellular interactions at the alveolar level.[12] BAL is generally well tolerated, even in these unwell patients and is lower risk procedure than in surgical lung biopsy.[12] In addition to the microbiological investigations, functional and imaging modalities are used to evaluate lung injury post HCT; however, these tools often diagnose lung disease once severe and potentially irreversible.

Following a diagnosis of post HCT lung disease, morbidity and mortality is significantly increased. Pulmonary complications are a leading cause of non-relapse mortality and even in those who survive initially, their 10-year mortality rates are higher than those who do not develop pulmonary complications.[13] These adverse patient outcomes are likely due to many contributing factors, including the incomplete understanding of the pathophysiology, as well as inadequate treatments of lung complications. Moreover, early and tailored therapy to target underlying mechanisms responsible for the lung injury are lacking. There is an urgent need for improved strategies to identify patients at risk of pulmonary complications before irreversible lung damage occurs, as well as rational and more effective therapeutic options for prevention and treatment. Biomarkers offer a potential strategy to assist in the prediction, prognostication and understanding of the biological mechanisms contributing to post HCT lung disease to improve patient outcomes.

METHODS: SEARCH STRATEGY

This review examines the current body of evidence related to biomarkers to predict the development of lung disease and/or its severity, diagnose and treat children with lung injury post HCT.

Medline, Embase and PubMed databases were searched using the search terms (child or paediatric) AND (haematopoietic stem cell transplant) AND (bronchoalveolar lavage OR

biopsy OR lung OR virus or bacterial or fungal infection). The inclusion criteria were studies that involved paediatric participants undergoing HCT and the exclusion criteria were animal studies and those that did not specifically measure a biomarker. Studies were also excluded if they measured only functional (lung function studies, e.g. Spirometry) or imaging biomarkers (e.g. Parametric response mapping on computed topography). The initial number of studies identified in the search were 2796. These were reviewed initially by title and abstract and reduced to 201. The reference lists of these articles were also reviewed for suitable articles. In total, 19 articles fulfilled inclusion and exclusion criteria. The results were summarised in a narrative review, given the heterogeneity of biomarkers did not permit a meta-analysis. The included articles were divided into categories of acute and chronic lung injury post HCT. Table 1 summarises these biomarkers and at which timepoint in relation to transplant they have been utilised in the studies included in this review. Figure 1 highlights the key biomarkers and associated diseases in acute and chronic pulmonary complications post HCT.

RESULTS

Acute lung injury

There have been 10 studies identifying 12 different biomarkers for the prediction and diagnosis of acute lung injury. The key findings of these studies are detailed in Table 2. Several researchers[12,14–16] focused on general acute lung injury as the primary outcome rather than a specific infectious or non-infectious diagnosis.

Neutropenia as a prognostic marker

There is evidence that neutropenia, both in the pre and post engraftment period, can be used as a biomarker for post HCT pulmonary complications. The aplastic phase post HCT is characterised by neutropenia. This commonly persists for the first 3 weeks post stem cell infusion and is associated with a high incidence of infective complications. A systemic absolute neutrophil count of less than 1×10^9 L^{-1}, within 48 h of developing lung imaging changes, has been associated with an increased risk of mortality. A single-centre study, including both infective and non-infective complications, demonstrated a higher risk of death in children who were neutropenic when they developed their pulmonary complication.[15] The duration and severity of neutropenia was not specified in this study as potential contributing factors. Although it is well known that prolonged and profound neutropenia significantly increases the risk of infective complications. A relatively deplete pulmonary neutrophil count, measured on BAL fluid, has also been shown to be a potential diagnostic tool in the post HCT setting.[16] A different single-centre study showed relative neutrophil depletion in addition to predominant lymphocytosis was seen in children post HCT who developed lung disease than in those who received chemotherapy alone.[16] In addition to conditioning agents, systemic inflammation, mediated by cytokines IL-6 and TNF, is associated with bone marrow suppression and the development of neutropenia post HCT.[15]

Cytokines for prediction of early respiratory failure and severe lung disease

Cytokines, the soluble mediators of inflammation, produced to mediate downstream cellular effects, have been shown to predict the development of post HCT lung disease.[12,14,17] Cytokines in these studies were measured by ELISA and sampled in both peripheral blood and BAL fluid. An example is IL-6, a pro-inflammatory cytokine released by macrophages, fibroblasts, Th17 and B cells.[12] Increased levels of IL-6 measured in both pre HCT BAL[12] and early post HCT peripheral blood[14] were associated with the development of post HCT lung disease and the development of acute respiratory failure post HCT suggesting IL-6 is a strong candidate biomarker for post HCT lung disease. In addition, ST2/IL-33receptor elevated in the first week post HCT has been associated with early development of respiratory failure.[14] This pathway has been implicated in a variety of inflammatory disorders as a mediator of type 2 inflammatory response and IL-33 receptors have also been found on lung epithelium.[18]

Cytokines as biomarkers for prediction of specific respiratory diseases post HCT have also been investigated.[19] Influenza is a common respiratory virus and is associated with increased morbidity and mortality in immunocompromised patients.[19] Severe influenza, defined by ICU admission or development of pneumonia, has been associated with a peripheral blood cytokine

Table 1. Summary of clinical timepoints and translation of biomarker evidence into potential therapeutic target

Collection timepoint related to HCT	Biomarker measurement site	Biomarker specific detail	Therapeutic strategy (with clinical evidence)	Potential therapeutic strategy[a]
Prior to HCT (Asymptomatic)	Peripheral blood *recipient*	*ACE* genotype		ACE inhibitors
		NOD2/CARD15 SNP variants		
	Peripheral blood *donor*	*NOD2/CARD15* SNP variants		
	BAL fluid	TNF		Etanercept
		GM-CSF		
		IL-1β		
		IL-8		
		G-CSF		
		Pulmonary microbiome analysis		
	Upper respiratory tract	Viral respiratory PCR swab	If positive for virus delay transplant	Prolonged immunosuppression to reduce allo-immune disease[b]
Asymptomatic; Day + 7 post HCT	Peripheral blood	ST2 (IL-33 receptor)		Tocilizumab
		IL-6		
Pulmonary symptoms present	Peripheral blood	TNF	Etanercept	Tocilizumab
		MCP-1		
		TNFR1		
		TNFRII		
		IL-6		
		sCD17		
		IL-8		
		Ang-2		
		ST2		
		LBP		
		Absolute neutrophil count		
	BAL fluid	Total Cell count (lymphocyte, neutrophil and atypical epithelial cell proportions)	Etanercept	Tocilizumab
		CCL2/MCP-1		
		TNF		
		MCP-1		
		TNFR1		
		TNFRII		
		IL-6		
		sCD17		
		LBP		
Influenza diagnosis	Peripheral blood	IFN-y		Emapalumab
		IL-13		
Late post HCT > 100 days post Day 0	Peripheral blood	Leucotriene receptor levels	Montelukast	
		TNFR1	Etanercept	
		TNFR2		
Imaging or lung function suspicious for BO or RLD		IL-8		
		TGF-β		
	BAL fluid	Pulmonary microbiome analysis		

BAL, bronchoalveolar lavage; BO, bronchiolitis obliterans; HCT, haematopoietic cell transplant; LBP, lipopolysaccharide binding protein; RLD, restrictive lung disease; TNF, tumour necrosis factor; TGF, tumour growth factor; GM-CSF, granulocyte macrophage colony stimulating factor; G-CSF, Growth colony stimulating factor.

[a]These have shown safety in children post HCT setting but require further targeted studies to determine efficacy for pulmonary complications post HCT.

[b]Increased immune suppression may increase risk of developing opportunistic infections.

profile of elevated IL-10 and decreased T helper 2 response[19] (lower IL-13 levels). These findings suggest that a T helper 2 immune response may be protective against severe influenza disease post HCT.[19] Usually, IL-10 is thought to be associated with a T helper 2 response; however, in this case,

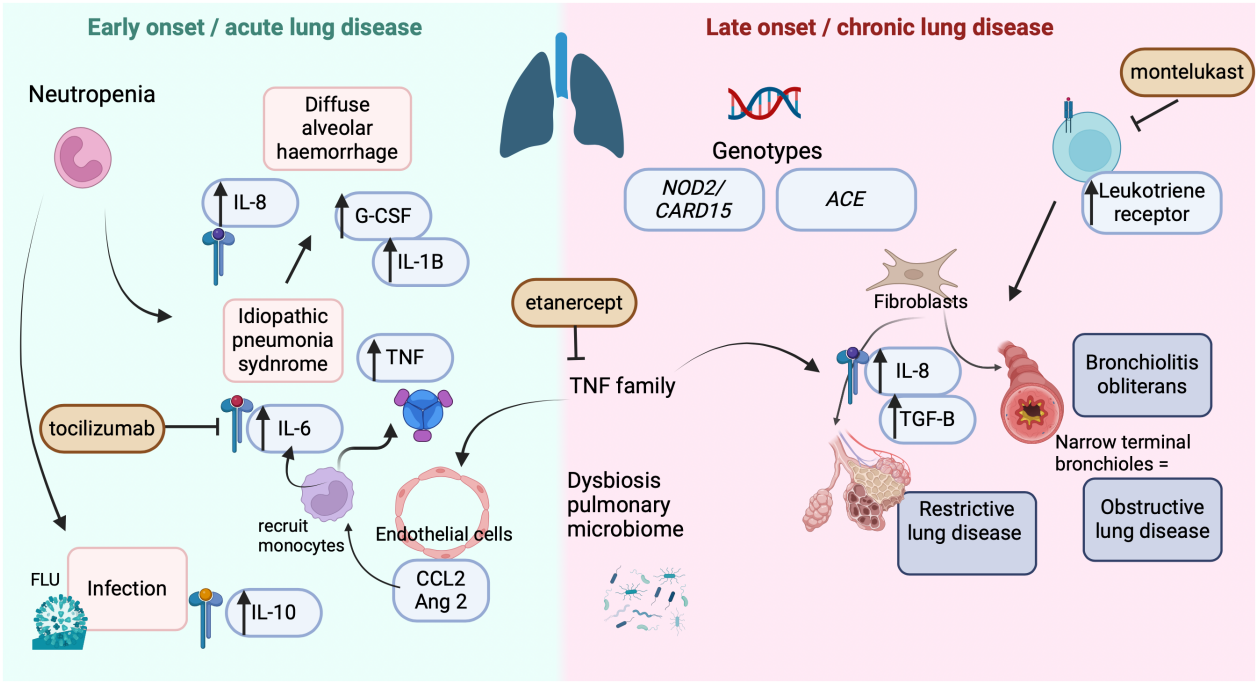

Figure 1. The key biomarkers and associated causes of acute and chronic pulmonary disease post-transplant. The crossover between the TNF family in causes of both acute and chronic lung disease is demonstrated. Therapeutic targets with clinical data supporting the potential use are also shown, for example, montelukast and etanercept. Biorender.com was used in the drafting of this figure. HCT, haematopoietic cell transplant; TNF, tumour necros is factor.

given the differing direction of association with influenza severity, it may be that IL-10 production is being driven by alternate cells.

Cytokines associated with idiopathic pneumonia syndrome

A severe manifestation of non-infectious or 'allo-immune' acute lung injury is IPS. IPS is one of the most widely investigated examples of a disease specific model for the use of clinical biomarkers post HCT and how these can be translated into targeted treatment. Several studies[20–22] have identified biomarkers of innate immunity, endothelial damage and systemic inflammation contributing to the development of IPS, with the strongest association being tumor necrosis factor alpha (TNF).[22,23] TNF is released following tissue damage secondary to conditioning agents such as total body irradiation (TBI) and in the setting of acute graft versus host disease (aGVHD).[24] Elevated levels of TNF and its soluble receptor TNFR1, measured in BAL fluid and plasma post HCT, in patients who develop IPS, have been demonstrated in a both a small discovery cohort[22] and larger paediatric cohort in comparison to controls.[23] C-C motif ligand 2 (CCL2) also referred to as MCP-1, monocyte chemoattractant protein, is released from endothelial cells in response to TNF and IL-1B.[21] MCP-1 functions to recruit monocytes and fibroblasts to sites of inflammation, for example, the lung in the case of IPS.[21] Elevated levels of MCP-1 in both BAL and plasma have also been implicated as a mechanistic biomarker in children with IPS than unaffected children.[21,22] Another mediator of endothelial activation and impending tissue damage is the protein Ang-2, which is expressed on endothelial cells and functions to sensitise cells to TNF.[23] Ang-2 has been shown to be raised in children with IPS in plasma at diagnosis and also to correlate with response to

Table 2. Summary of research biomarkers investigating acute lung injury

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Hildebrandt *et al.* 2004[21]	BAL fluid at the time of developing pulmonary complication	Prospective, single centre *N* = 30 Adult and paediatric	CCL2/MCP-1 ELISA	IPS defined as diffuse lung injury in the first 100 days post HCT in the absence of infection	11 patients who developed IPS had significantly elevated CCL2/MCP-1 levels ($P < 0.05$) in BAL fluid than patients with chronic post HCT lung disease ($n = 14$) and normal controls ($n = 5$)
Yanik *et al.* 2008[22]	BAL fluid and peripheral blood	Prospective, multicentre Patients diagnosed with IPS *N* = 15 Adult and paediatric Control groups BAL cytokines $n = 16$ and plasma cytokines $n = 16$	TNF MCP-1 TNFR1 TNFRII IL-6 sCD17 LBP ELISA	Response to etanercept, defined as time to decreased oxygen requirement and survival at 28 days Response of inflammatory cytokines; systemic and pulmonary to treatment	Response to etanercept was seen in 13/15 patients. Survival at day 28 was 73% Elevated BAL and plasma levels in patients with IPS than controls; TNF, TNFR1, IL-6, sCD14, MCP-1 (CCL2) $P < 0.01$
Yanik *et al.* 2015[23]	Peripheral blood at onset of IPS and weekly for 4 weeks	Multicentre Phase II; prospective Trial *N* = 28 Paediatric Control groups $n = 9$ (combination of healthy patients and children post HCT without lung complications)	IL-8 IL-6 Ang-2 LBP sTNFR1 sTNFR2 LBP ELISA	Response to etanercept, defined as time to ceasing oxygen therapy and survival at day 28 and 1 year. Plasma inflammatory cytokines	Response in 71% (20/28) to etanercept Overall survival at 28 days 89% (CI 70–96) and 1 year 63% (CI 42–79%) TNFR1, used as a surrogate for TNF was increased in plasma of patients at IPS dx $P < 0.001$ than control group Significantly higher levels of; IL-6, IL-8, Ang-2, LPB and sTNFR2 at diagnosis of IPS $P < 0.01$ Responders to etanercept showed decline in plasma sTNFR1, IL-8 and Ang-2 than non-responders, not statistically significant
Whittle *et al.* 2001[12]	BAL fluid containing alveolar macrophage population pre HCT and post HCT	Prospective, single centre *N* = 34 adult & children Comparing patients with ($n = 11$) and without ($n = 21$) post HCT lung disease	TNF GM-CSF IL-6 ELISA Cytokines reported as a proportion of AM in cell culture	Post HCT lung disease defined as new radiographic opacification, or sustained tachypnoea with a fall in oxygen saturation > 5% from baseline on two readings, within 6 months from HCT	Median increase in cytokine levels in post HCT lung dx group than patients who did not develop pulmonary complications • TNF: 3 ng 10^{-6}AM (95% CI 0.1–7), $P = 1.01$ • GM-CSF: 4 ng 10^{-6} AM, $P = 0.006$, 95% CI = 0.5–7 IL-6: 0.3 ng 10^{-6} AM, CI 0.0002–1 $P = 0.049$
Kharbanda *et al.* 2006[17]	BAL fluid pre HCT	Prospective single site, *N* = 48 children, MPS Comparing patients with ($n = 25$) and without ($n = 23$) lung disease	IL-1β IL-8 G-CSF ELISA	Post HCT lung complications defined as either infectious or non-infectious by two study investigators based on clinical, radiological and bronchoscopic findings	Higher (mean) cytokine levels in post HCT lung dx group than the children who do not develop pulmonary complications post HCT • IL-β (104.2 picogramper g total protein versus 69.9 $P = 0.69$), • IL-8 (4327.2 versus 3155.7, $P = 0.052$) • GCSF (1022.3 versus 397.0 $P = 0.16$)

(Continued)

Table 2. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Rowan *et al.* 2018[14]	Peripheral blood post HCT, Day + 7	Retrospective single centre, n = 122 (63 children) Comparing n = 22 patients who developed respiratory failure to n = 100 who did not post HCT	ST2 (IL-33 receptor) IL-6 ELISA	Respiratory failure within 30 days post HCT	Elevated ST2 (IL-33 receptor) and IL-6 at day 7 post HCT was associated with acute respiratory failure within 30 days, OR 8.9 (2.8, 28.6) (95% CI, $P < 0.001$) and 11.9 (2.6, 53.8) (95%CI, $P < 0.01$)
Choi *et al.* 2017[15]	Peripheral blood within 48 h of respiratory infiltrates on imaging post HCT	Retrospective observational, single site N = 35 children	Neutropenia	Mortality due to post HCT lung disease (up to 3 years post HCT) Pulmonary complication defined as the pulmonary infiltrates on chest imaging simultaneously with respiratory symptoms requiring admission, evaluation, or treatment	ANC $< 1 \times 10^9$ L^{-1} (OR 10.5, 95% CI, 1.07–104.65, $P = 0.044$) was associated with increased mortality post HCT
L'Huillier *et al.* 2019[19]	Peripheral blood post HCT at the time of Influenza diagnosis	Multicentre prospective observational study N = 277 (both paediatric and adult) Comparison of serum levels in n = 277 patients at two timepoints post HCT	Luminex 100 system Th1: IFN-y Th2: IL-13	ICU admission or development of pneumonia related to Influenza post HCT	Lower Th1:Th2 ratio at Day 0 associated with reduced ICU and at Day 28 associated with reduced ICU admission and pneumonia development. $P < 0.05$
Rochat *et al.* 2008[16]	BAL in symptomatic patients post HCT	Retrospective case control, single centre N = 19 Comparing n = 9 children post HCT to n = 10 children who received chemotherapy alone and developed pulmonary complications	Lymphocyte count Total and differential cell count; presence of cellular atypia Differences in the mean percentage cellular composition patterns between the two groups	Respiratory complications defined as rapidly progressing respiratory symptoms and radiological findings (new or persistent infiltrates on CXR or CT)	Post HCT pulmonary disease group than the chemotherapy alone group had • Predominant lymphocytosis (18% versus 6.25%, $P = 0.03$), • Higher levels of atypical epithelial cells (75% versus 30.8%, $P = 0.027$) • Neutrophil deplete (25.9% versus 58%, $P = 0.02$)
Seo *et al.* 2018[20]	Peripheral blood post HCT in patients with pneumonia/IPS/ Controls	Retrospective Single centre, case control N = 41 children and adults Comparing 41 patients who developed IPS to a cohort of n = 162 controls (no infection post HCT) and n = 37 who developed viral infection	ELISA IL-6 ST2 TNFR1	Diagnosis of Idiopathic pneumonia syndrome than viral pneumonia	Elevated ST2 and IL-6 in IPS post HCT versus controls OR 2.8 (2.0–4.0, 95% CI, $P < 0.001$) and 1.4 (1.0–1.9, 95% CI, $P < 0.025$) Elevated TNFR1 in IPS than viral pneumonia at onset of respiratory symptoms, OR 2.9 (1.5–6.0, 95% CI, $P < 0.001$)

AM, alveolar macrophages; BAL, bronchoalveolar lavage; HCT, haematopoietic cell transplant; IPS, idiopathic pneumonia syndrome; LBP, lipopolysaccharide binding protein; MPS, mucopolysaccharidoses.

targeted therapy, with etanercept.[23] Etanercept, a soluble TNF inhibitor, previously used in other inflammatory conditions such as rheumatoid arthritis, was trialled as a treatment for IPS.[22] A paediatric multicentre phase II trial showed clinical response in 71% of children treated with etanercept, when used in combination with corticosteroids, with overall survival of 63% at 1 year.[23] This is compared to historical cohorts prior to etanercept which reported survival of only 20–50% in patients who develop IPS post HCT.[25,26] Similar to general acute lung injury, IL-6 has also been shown to have specificity to IPS as a diagnostic biomarker[20,22,23] at the time of developing respiratory symptoms post HCT. Overall, IPS is a landmark example of how diagnostic biomarkers can not only be utilised for therapeutic targets but also highlights the need for ongoing strategies to improve sustained survival in non-responders.

Diffuse alveolar haemorrhage

A subset of IPS, DAH is a rare complication post HCT but is associated with high mortality.[17] DAH has been observed at higher rates in children with a diagnosis of metabolic storage disorders such as mucopolysaccharidoses (MPS), making them a cohort of particular need of predictive biomarkers.[17] Elevated levels of IL-1B, IL-8 and G-CSF in bronchoalveolar fluid of children pre HCT was seen in the group of children who developed post HCT lung complications, including DAH which occurred in 19% of these children.[17] IL-1B IL-8 and G-CSF are pro-inflammatory cytokines involved in T cell stimulation, chemotaxis and activation of monocytes and neutrophils.[17] This suggests an increased risk signature pre HCT in this group of children, who are relatively 'treatment naïve' in comparison to their counterparts who undergo HCT for a malignant indication.

Chronic lung injury

There have been nine studies exploring eight different biomarkers for the prediction and diagnosis of chronic lung injury. The key findings of these studies are detailed in Table 3.

Bronchiolitis obliterans

One of the most common types of non-infectious chronic lung disease post HCT is BOS, a pulmonary manifestation of chronic graft versus host disease (cGVHD).[27] BOS is diagnosed in adults using pulmonary function tests to demonstrate obstructive lung disease (OLD) and imaging studies showing air trapping, in the absence of infection.[28,29] In children, pulmonary function testing is more challenging and often not reproducible or possible in those aged less than 7 years.[30] This is problematic as BOS is often asymptomatic until significant airflow obstruction has occurred and conversely once symptomatic the differential diagnosis is broad, including infection and alternative lung pathology. This highlights the need for alternative and complementary biomarkers for diagnosis of BOS.

Leucotriene receptor levels have been shown in adult and paediatric populations to be elevated in both the peripheral blood and urine of patients who have been diagnosed with BOS, than healthy controls.[31] Leucotrienes are responsible for mediating multiple immune pathways; of relevance in BOS this is thought to be related to activation of fibroblasts, leading to collagen deposition and obstruction of terminal bronchioles.[31] This led to the use of montelukast therapy, which blocks this pathway, for treatment of BOS. A Phase II trial[31] of patients treated with montelukast therapy showed a decrease in leucotriene receptor levels in peripheral blood monocytes and neutrophils and functional improvement with slower decline of forced expiratory volume in 1 s (FEV1). Whilst this was a small cohort of patients, it is an example of the translation of a biomarker to a therapeutic target and correlation to functional studies (spirometry).

Restrictive lung disease

Restrictive lung disease can also be a manifestation of chronic GVHD, with TBI being a known risk factor.[32] In contrast to OLD, RLD post HCT is associated with reduced forced vital capacity (FVC) and total lung capacity (TLC). Whilst OLD and RLD differ in terms of functional assessment and pulmonary imaging, similar patterns of elevated plasma biomarkers (TNFR1, TNFR2, IL-8 and TGF-B) have been observed post HCT than patients without chronic lung disease.[33] This suggests that cGVHD culminating in end-stage fibrosis have shared biomarker profiles despite different anatomical involvement, that is, in terminal airways in OLD than the interstitium in RLD. As a result, etanercept, was trialled as a

Table 3. Summary of research biomarkers investigating chronic lung injury

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Versluys *et al.* 2018[40]	Paired BAL and NPA pre HCT	Retrospective Single centre *N* = 179 children Comparing patients with respiratory virus *n* = 110 to *n* = 69 who did not have a respiratory virus pre or post HCT	Viral respiratory panel compared NPA to BAL findings PCR assays	Post HCT lung complications, defined as Idiopathic pneumonia syndrome or bronchiolitis obliterans as defined by the American Thoracic Society; IPS = evidence of widespread lung injury by clinical symptoms and radiological abnormalities in the absence of LRTI and other factors to explain pulmonary dysfunction (cardiac dysfunction, fluid overload or renal failure) BOS = NIH 2014; FEV1/VC ratio of < 0.7, FEV1 of < 75% and evidence of air trapping (on PFTs or HRCT)	Virus detected pre HCT on BAL predictive of alloimmune lung disease: HR, 3.8 (95% CI, 1.4–10.7, *P* = 0.01) Virus detected on pre HCT NPA was not predictive of post HCT lung disease
Versluys *et al.* 2010[39]	NPA swabs post HCT	Prospective single centre *N* = 110 children	Respiratory virus PCR on NPA	Development of alloimmune lung disease acute (IPS) or chronic (BO/BOOP) allo lung dx	Respiratory virus infection early post HCT was predictive of allo-immune lung disease (*P* < 0.0001) BOS; HR = 107; 95% CI (0.9–13 347, *P* = 0.05) IPS; HR = 11.4; 95% CI (2.61–49.8 *P* = 0.01) Grade II-IV aGVHD post HCST was protective in relation to development of allo-immune lung disease
Williams *et al.* 2022[31]	Peripheral blood, urine and BAL	Phase II, prospective multi centre *N* = 23 s mod-severe lung disease Adult and paediatric Compared to historical cohort of healthy patients	Leucotriene receptor levels on blood eosinophils and monocytes and BAL at enrolment Flow cytometry on whole blood Mass spectrometry on BAL	To assess whether montelukast altered lung decline for patients with established BOS FEV1 stability or improvement (≤ 15% decline) at 6 months of montelukast therapy Leucotriene receptor activity	Elevated leucotriene receptor levels on blood eosinophils (*P* = 0.018) and monocytes (*P* = 0.027) in BOS patients versus healthy controls. These decreased with treatment to montelukast therapy Leucotriene receptors were not detected in BAL samples 91% of (21/23) patients met criteria for FEV1 stability or improvement after 6 months of therapy
Miyamoto *et al.* 2014[35]	Peripheral blood pre HCT	Retrospective single centre *N* = 149 (adult and children) Comparing *n* = 18 who developed non-infectious pulmonary complications and *n* = 131 who did not develop NIPC post HCT	*ACE* genotype ACE enzyme PCR amplification & ELISA	Development of non-infectious pulmonary complications post HCT NIPC was defined as evidence of widespread alveolar injury in multilobar infiltrate, and clinical symptoms of pneumonia and evidence of abnormal respiratory physiology, for example, impairment on pulmonary function test	*ACE* D/D (deletion/deletion) genotype, increased risk of developing non-infectious pulmonary complications post HCT, HR 8.8, *P* < 0.001

(Continued)

Table 3. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Yanik *et al.* 2012[33]	Peripheral blood	Prospective, single centre Patients with obstructive or restrictive lung dx post HCT *N* = 34 Comparing to controls post BMT Adult and paediatric	Plasma cytokine levels ELISA	Primary outcome: Response to etanercept related to FEV1 or FVC improvement (10% increase) at 4 weeks To determine plasma biomarkers for diagnosis and tracking therapy response to etanercept	33% of patients met primary outcome, improvement in lung function at 4 weeks but this was not statistically significant $P = 0.73$ Elevated TNFR1, TNFR2, IL-8, *TGF-β* in plasma of patients with OLD or RLD
Zinter *et al.* 2024[37]	BAL fluid at the time of pulmonary complication post HCT	Prospective, multicentre *N* = 229 children 278 BAL samples collected at the time of pulmonary complication; underwent analysis of pulmonary microbiome, immune cell gene expression and mortality Validation cohort 57 children from the Netherlands	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells	Key outcomes; Patient cluster and overall survival Patient subtypes classified as; Cluster 1: High diversity of commensal microbiota, predominance of antigen presenting cells (alveolar macrophage) Cluster 2: Increase in bacterial organisms, increased neutrophil infiltration and activation Cluster 3: Depletion of commensal microbes, increased viral and fungal pathogens, higher proportion of lymphocytes with activation of fibroproliferative pathways Cluster 4: Depletion of commensal microbes, increased S. aureus and viral pathogens, higher proportion of lymphocytes	4 key clinicopathological clusters of patient subtype based on BAL sample analysis were identified and prognostic of patient survival Diverse pulmonary microbiome was the most commonly seen (Cluster 1) associated with the lowest mortality ($P = 0.005$) Deplete pulmonary microbiome and enrichment of viral pathogens (Cluster 3 &4); were associated with higher mortality ($P = 0.005$)
Zinter *et al.* 2022[11]	BAL fluid and paired pulmonary function tests pre HSCT in asymptomatic children	Prospective single centre *N* = 104 children, the Netherlands	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells PFT grouping related to lung capacity, obstruction, diffusion and air trapping	To determine if there is an association between pulmonary microbiome and PFTs patterns, for example, obstruction or restriction pre HSCT and all-cause mortality post HCT	Reduced FVC% pred was associated with post HCT mortality, HR 1.21(1.03–1.43, $P = 0.024$) 95% CI Multivariate analysis; Reduced lung capacity, pre HSCT is associated with a pulmonary microbiome that is deplete, suggest a pro-fibrotic immune signature and predicts higher post HCT mortality
Zinter *et al.* 2021[36]	BAL fluid collected pre HSCT	Retrospective, single centre *N* = 181 children, the Netherlands 181 BAL samples for analysis collected prior to HCT as part of clinical care for another reason, for example, Central line insertion in children who did not have clinical suspicion for a lung infection	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells	Key outcomes: Development of post HCT lung injury Non-relapse mortality and fatal lung injury BAL metatranscriptome clusters: Cluster 1: Oropharyngeal heavy (microbiota rich) Cluster 2: Microbially deplete Cluster 3: Oropharyngeal light Cluster 4: Virus enriched	Lung injury Microbially deplete and virus enriched microbiome associated with increased development of post HCT lung injury; HR 95% CI 5.6 (2.5–12.8, $P < 0.001$) and 3.5 (0.9–13, $P = 0.06$) Fatal Lung Injury

(Continued)

Table 3. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
		Validation cohort 18 children; underwent BAL for a clinical indication			Microbially deplete and Virus enriched microbiome associated with increased fatal lung injury; HR 95% CI 6.8 (1.9–24.3, $P = 0.003$) and 6.3 (1.1–38.2 $P = 0.044$)
Hildebrandt *et al.* 2008[28]	Peripheral blood	Multicentre, prospective $N = 427$ Adult and paediatric	Blood of both donor and recipient pairs, DNA, SNP array	Development of BO and differences with specific donor/recipient *NOD2/CARD15* SNP mutations than wildtype BO defined using NIH criteria	Recipient or donors with *NOD2/CARD15* SNP variants (SNP8, SNP12, SNP13) are associated with increased development of BO in 18.7% ($P < 0.001$) than 1.3% incidence of BO without these mutations (wildtype) Recipient mutations had a stronger association than donor mutations in multivariate analysis

aGVHD, acute graft versus host disease; AM, alveolar macrophages; BAL, bronchoalveolar lavage; BO, bronchiolitis obliterans; BOS, bronchiolitis obliterans syndrome; FEV, forced expiratory volume; FVC, forced vital capacity; HCT, haematopoietic cell transplant;HRCT, High resolution Computed topopgraphy; HSCT, Haematopoietic stem cell transplant; IPS, idiopathic pneumonia syndrome; LBP, lipopolysaccharide binding protein; MPS, mucopolysaccharidoses; NIH, National institute of health; NPA, Nasopharyngeal aspirate; OLD, obstructive lung disease; PFT: Pulmonary function test.

therapeutic target and while a small cohort of patients demonstrated improvement in both obstructive and restrictive patterns of spirometry, there was no statistically significant difference in overall survival.[34]

Genotypes to predict risk of chronic lung disease

In addition to soluble biomarkers, two studies[28,35] have identified genetic variations that may influence the development of lung disease post HCT, in angiotensin converting enzyme (ACE) and nucleotide binding oligomerisation domain containing 2/caspase recruitment domain family, member 15 (*NOD2/CARD15*). The ACE enzyme is active in lung endothelium and functions to produce Angiotensin II, a protein that acts on pulmonary fibroblasts, shown to contribute clinically to pulmonary hypertension and fibrosis.[35] Specific variations in the *ACE* genotype have been associated with an increase in non-infectious lung disease post HCT (obstructive and restrictive).[35] By comparison, the *NOD2/CARD15* gene is expressed on lung epithelial cells, as well as circulating monocytes and macrophages.[28] Mutations in the *NOD2/CARD15* gene were associated with a statistically significant higher incidence of BOS development than patients without this mutation (wildtype).[28] Both studies[28,35] identified patients with genetic biomarkers that, via different pathways, lead to alloreactive immune dysregulation toward a common pathway of pulmonary fibrosis.

Pulmonary microbiome pre and post HCT

There have been three studies from the same group that explored the relationship with pulmonary microbiome and chronic lung disease in paediatric HCT. The ability to describe patterns of pulmonary microbiome signature or 'cluster', on BAL samples, has been shown to have potential as a biomarker both pre and post HCT for the development of post HCT lung injury.[11,36] The finding that the lung, similarly to the gastrointestinal tract, is not sterile and host to wide array of micro-organisms, has been demonstrated in several paediatric studies.[11,36,37] Initially identified in a retrospective cohort,[36] it was shown that children with pulmonary microbiome clusters pre HCT that were either deplete of commensal microbiota (cluster 2) or

viral enriched (cluster 4) had higher rates of post HCT lung injury and subsequent death. A subset of these children also had matched pre HCT pulmonary function tests performed.[11] Poor pulmonary function pre transplant has been shown to be independently associated with worse post HCT outcomes in terms of non-relapse mortality.[38] The correlation of pulmonary microbiome data with pulmonary function tests identified an abnormal microbiome, defined as deplete of common commensal organisms, was associated with reduced lung capacity (FVC% pred).[11] The immune profile of the BAL fluid in these patients with microbiome dysbiosis was also associated with a pro-fibrotic immune response, which may have contributed to the lower lung capacity seen in these children.[11] A prospective trial from the same group Zinter *et al*.[37] applied similar analysis techniques in children post HCT, with BAL samples collected to investigate a pulmonary complication. Again, this identified that a diverse pulmonary microbiome was protective and associated with lower mortality post HCT than a viral enriched, deplete microbiome.[37] A cohort of children in this study also required a second BAL for new or progressive pulmonary disease and this revealed a temporal shift of the microbiome to cluster as high risk.[37] This suggests ongoing pulmonary insults post HCT continue to influence the microbiome. Collectively these three studies[11,36,37] of the pulmonary microbiome demonstrate that microbiome dysbiosis may be associated with an increased risk of developing chronic post-transplant lung disease and increased mortality.

Influence of viral infection on chronic lung disease

Similar to the finding that a pulmonary microbiome enriched with viral organisms is associated with worse outcomes, the presence of viral infection, detected using quantitative PCR methods, has a role as a predictive biomarker for post HCT lung disease.[27,39,40] Respiratory virus pathogens detected on BAL fluid pre HCT[40] and nasopharyngeal swab early post HCT[39], have been shown to increase a child's risk of post HCT chronic lung disease.[40] This included viruses that did not cause significant or severe lung disease with initial infection, for example, Rhinovirus, highlighting the potential pathogenic role of these infections in promoting donor-mediated allo- reactive immune dysregulation. To understand the link between the presence of a virus and clinical outcome post HCT, more research is required into the individual cytokine response or gene expression profiles of the patients who have worse clinical outcome.

DISCUSSION

This review identified 19 studies that investigated the use of predictive, diagnostic and therapeutic biomarkers in children who develop post HCT lung complications. Within these studies, over 20 individual biological biomarkers in blood and BAL fluid were investigated. Cytokines, soluble mediators of inflammation and infection, were commonly identified as biomarkers for both acute and chronic lung complications. Broadly, interactions between cytokines and their receptors, contribute to leucocyte migration and are key to both adaptive and innate immune responses.[21] It is not surprising that many of the studies identified in this review investigated cytokines as key candidate biological biomarkers. In the case of HCT, the cellular immune responses become more complex due to the nature of transplant chimera. Specifically, the interactions between donor and recipient immune cell populations and the actions of cytokines produced by both the donor and recipient. In relation to pulmonary complications, it is plausible that the lung resident immune cells, for example alveolar macrophages, are influenced by a milieu of systemic cytokines from the donor as well as by recipient innate cells within lung tissue. One of the potential limitations of several studies reviewed is the difficulty in delineating the cellular 'source' of cytokine production, specifically, donor versus recipient. Over time, post HCT, the expanding immune cell populations from donor origin predominate, particularly when chimerism is $> 95\%$. This may be important to understand in the context of time post HCT and degree of immune reconstitution of the donor. There are many factors, including those between host and recipient that contribute to dysregulated inflammation in response to cumulative tissue injury from HCT.

It is well established that the development of lung complications posts HCT contribute significantly to paediatric intensive care unit admission and non-relapse mortality post HCT.[41] Several authors looked at pre HCT cytokines as

predictive biomarkers of mortality post HCT. The pre HCT cytokine levels in this setting would be produced by the patient (recipient) who has not yet received a HCT and suggests some predisposition that is potentially independent of the donor. Common cytokine patterns identified pre HCT that were predictive of post HCT disease were elevated IL-6 and GM-CSF levels.[12,14,20,22,23] In contrast, in the post HCT setting, the cytokine milieu is likely to be produced and influenced to a greater proportion by the engrafted donor immune cell population. In the lung, however, like other extramedullary organs, there is potentially more heterogeneity in the immune populations from the donor and recipient. Perhaps, it is this complex cellular interplay within the lung, which makes this organ a key target for the spectrum of post HCT complications observed in our patients.

The relationship between immune mechanisms in the lung post HCT has been extrapolated from patients who have required lung transplantation for severe BOS.[42,43] In patients who required a lung transplant for severe BOS post HCT, proinflammatory M1 alveolar macrophages of stem cell donor origin were the dominant immune cell type found in the explanted lungs of patients who underwent lung transplantation for post HCT BOS[42]. This is in comparison to a clinically similar phenotype of BOS post lung transplant in which the solid organ (lung) *recipient* alveolar macrophages were the dominant cell type responsible.[42] This highlights that both types of BOS share a similar clinical phenotype and pathophysiology, in both examples, the alveolar macrophage is of a different patient origin to the lung. It is likely that in future more can be learned from this patient group to design future biomarker studies in the paediatric post HCT cohort.

It is important to highlight that many of the studies identified in this review measured cytokines in cohorts of both adults and children undergoing HCT. This strategy, while allowing larger populations to be studied and improve statistical power, assumes that these populations are comparable. It is critical to note that key differences between children and adults in relation to immune physiology, transplant regimens and indications for transplant. These differences limit the applicability of biomarkers measured in a 'mixed' cohort unless validated in an exclusively paediatric cohort. Key differences in the HCT setting in paediatrics include the primary indication for HCT, intensity of conditioning regimens, cell graft source and types of pre-existing lung disease.[44] Children, compared to adults, have a higher percentage of non-malignant indications for HCT, such as inborn errors of immunity, metabolic disorders and haemoglobinopathies.[45,46] There is also an increased use of myeloablative-level conditioning and preference for grafts from bone marrow or cord blood in children. In contrast, adults are more likely to receive reduced intensity conditioning regimens and peripheral blood grafts. These differences may explain disparate treatment response between adult and paediatric cohorts in clinical trials for some lung complications, for example etanercept in IPS. It is also important to also acknowledge that children are not small adults with respect to their immune systems. In healthy individuals, immune cell proportions change with increasing age, including increased memory/naïve T cell ratio and decreased T cell repertoire related to thymic involution age.[47] These differences might explain contrasting clinical severity of infections with a notable example being less severe COVID-19 disease in children than adults.[48] This is a noteworthy principle when considering the applicability of immune cell proportions and cytokine profiles in adults as biomarkers in children. Robust knowledge translation also requires the establishment of normal reference ranges in adults and children pre HCT and post HCT.

This review also highlighted significant overlap in biomarkers, such as IL-6 and TNF family, across acute and chronic lung complications. This raises the question of whether these complications are not distinctly separate entities but a spectrum with shared biomarker patterns in response to similar pathophysiology. For example, tissue damage locally (both endothelial and interstitial), leading to cytokine release and T cell influx with dysregulated immune response. An example of this is the connection between acute GVHD, chronic GVHD and BOS. While it is established that aGVHD is a risk factor for cGVHD, the latter only occurs in a subset of children with prior aGVHD.[49] By comparison, BOS can occur in children without other manifestations of GVHD and this is poorly understood.[49] It is plausible that specific host and donor factors contribute to the unique end-organ vulnerability of the lung to the immune dysregulation of cGVHD. Furthermore, there is significant variability in the clinical response of patients with BOS to

treatment, with some patients failing systemic immunosuppression and progressing to lung transplantation than patients who have resolution of BOS who require minimal systemic therapy. Genetic biomarkers, such as variants in *NOD2/CARD15*, may help predict risk and are plausible contributory factors in severe and treatment refractory BOS.

At the time of this review, there were no published clinical data trialling the use of IL-6 inhibition, for example, tocilizumab in the treatment of post HCT lung complications. There is, however, a precedent for the use of tocilizumab for the treatment of both severe COVID-19 and cytokine release syndrome post CAR-T therapy, making it a rational targeted treatment with plausible benefit to study prospectively (ideally in randomised fashion) to prevent and/or treat post HCT lung disease.

The influence of the pulmonary microbiome in the development of post HCT lung disease holds significant promise in better understanding the variability of clinical outcomes seen.[11] This work by Zinter *et al*.[11,36,37] highlights the importance of identifying not only pathogenic but protective microbiome environments that correlate with the maintenance of lung health. These may then guide future therapeutic strategies, akin to those directed at the intestinal microbiome post HCT. The microbiome of the gastro-intestinal tract pre HCT has been shown to strongly influence rates of post HCT GVHD and non-relapse mortality.[50] Zinter *et al*.[11,36,37] have demonstrated in multiple cohorts that the pulmonary microbiome both pre and post HCT can similarly predict the development of pulmonary complications and associated non-relapse mortality. Further studies are required to identify potential strategies to manipulate the pulmonary microbiome pre HSCT in those with high-risk profiles. By comparison, there is less known about how to best recreate a 'healthy' or diverse pulmonary microbiome. It is likely, however, that much can be learnt from gastrointestinal microbiome research, including targeted immunotherapy (e.g. tocilizumab) and faecal microbiota transplant.[51,52] The finding that the pulmonary microbiome BAL clusters also evolved into high-risk groups in patients who had persistent pulmonary disease suggests a progressive natural history.[37] The relationship between the oropharyngeal, lung and gut microbiome has been extensively investigated outside the HCT setting and dysbiosis has been shown to be associated with increased inflammation and opportunistic infection.[53] How the 'gut-lung' axis is impacted by HCT and can be used as a biomarker for prediction and treatment of lung complications is an area of ongoing research.

Now is an ideal time to profile the inflammatory imprint of lung disease due to increasing experience with targeted immunotherapy to treat lung diseases than ever before. Key examples are the use of tocilizumab to treat severe COVID-19 lower respiratory tract infections, due to the finding of elevated IL-6 in BAL fluid[54,55] and biologics targeting IL-4 including dupilumab for severe Asthma.[56]

CONCLUSION

Pulmonary disease continues to contribute significantly to morbidity and mortality for children post HCT.[1,41] This review highlights the wide range of potential biomarkers to aid in the prediction, diagnosis and prognosis of children who develop these complications post HCT. The development of a biomarker that can translate into improved clinical outcomes for patients requires multiple steps from discovery to implementation, including validation and verification. The implementation of the most promising biomarkers in a time efficient and cost-effective manner requires incorporation into large multicentre prospective clinical trials. Many of the non-infectious post HCT pulmonary complications that occur are not well understood, have limited targeted therapeutic options and consequently poor outcomes. Biomarkers incorporated into clinical practice are key to helping us identify new therapeutic targets and hence improve survivorship for children undergoing allogeneic HCT. The use of a 'panel' of multiple biomarkers, using a range of methods, for example, BAL, peripheral blood, imaging and functional analysis, will best fulfil the requirements for this complex and heterogeneous group of patients rather than a single biomarker. A multidisciplinary group of experts highlighted this, calling for the identification of pathobiology-based biomarkers of BOS which can detect BOS in its earliest stages, prior to changes in lung function or lung imaging.[57] Overall, this review has shown that many potential candidate biomarkers have been identified but ongoing research is required to determine those that will meaningfully translate into clinical care to improve outcomes for our patients.

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AUTHOR CONTRIBUTIONS

Hannah Walker: Conceptualization; data curation; writing – original draft; writing – review and editing. **Gabrielle M Haeusler:** Conceptualization; supervision; writing – review and editing. **Theresa Cole:** Conceptualization; supervision; writing – review and editing. **Melanie Neeland:** Conceptualization; supervision; writing – review and editing. **Diane Hanna:** Conceptualization; supervision; writing – review and editing. **Shivanthan Shanthikumar:** Conceptualization; supervision; writing – review and editing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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