Received: 2 February 2018 | Accepted: 6 June 2019

DOI: 10.1002/ppul.24456

ORIGINAL ARTICLE: INFECTION AND IMMUNITY

Long-term morbidity of respiratory viral infections during chemotherapy in children with leukaemia

Beryl Lin MD[1,2] | Brendan Kennedy BSc[2] | Jamie McBride GradCertPRS[3] | Luciano Dalla-Pozza MBBS[4] | Toby Trahair MBBS, PhD[1,5] | Geoffrey McCowage MBBS[4] | Emma Coward BClinExerPhys[2] | Leanne Plush MClinExPhys[3] | Paul D. Robinson MBChB, PhD[2,6] | Kate Hardaker PhD[2,6] | John Widger MD[1,3] | Anthea Ng MSc[4] | Adam Jaffe MD, PhD[3] | Hiran Selvadurai MBBS, PhD[2,6]

[1]Faculty of Medicine, University of New South Wales, Sydney, Australia

[2]Department of Respiratory Medicine, The Children's Hospital at Westmead, Sydney, Australia

[3]Department of Respiratory Medicine, Sydney Children's Hospital, Sydney, Australia

[4]Cancer Centre for Children, The Children's Hospital at Westmead, Sydney, Australia

[5]Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia

[6]Discipline of Paediatrics and Child Health, University of Sydney, Sydney, Australia

Correspondence
Hiran Selvadurai, Department of Respiratory Medicine, The Children's Hospital at Westmead, Locked bag 4001, Westmead, NSW 2145, Australia.
Email: hiran.selvadurai@health.nsw.gov.au

Abstract

Background: Respiratory viruses are a common cause of infection in immunosuppressed children undergoing cancer therapy. Pulmonary sequelae have been documented following respiratory viral infections (RVIs) in hematopoietic stem cell transplant (HSCT) recipients; however potential late effects in children undergoing nonmyeloablative chemotherapy have not been investigated.

Aim: To evaluate the long-term pulmonary morbidity of respiratory viral infections during chemotherapy in children with acute lymphoblastic leukemia (ALL).

Methods: Childhood ALL survivors, aged 7 to 18 years, greater than 6 months posttreatment were recruited. Exclusion criteria included HSCT or proven bacterial/fungal respiratory infection during treatment. Subjects were classified into "viral" or "control" groups according to retrospective medical records that documented the presence of laboratory-proven RVIs during chemotherapy. Symptom questionnaires (Liverpool, ISAAC) and lung function testing (spirometry, plethysmography, diffusing capacity, forced oscillation technique to ATS/ERS standards) were then performed cross-sectionally at the time of recruitment.

Results: Fifty-four patients (31 viral, 23 control) were recruited: median (range) age 11.2 (7.2-18.1) years, and at 4.9 (0.5-13) years posttherapy. Abnormalities were detected in 17 (31%) individuals (8 viral, 9 control), with the most common being DLCO impairment (3 viral, 4 control) and reduced respiratory reactance at 5 Hz (5 viral, 6 control). Children with RVIs during chemotherapy reported more current respiratory symptoms, particularly wheeze (odds ratio [OR], 3.0; 95% confidence interval [CI]: 0.9-10.0; $P = .09$) and cough (OR, 2.7; 95% CI: 0.8-9.5; $P = .11$). No differences in lung function tests were observed between the two groups.

Conclusions: Our study found children with RVIs during chemotherapy developed more long-term respiratory symptoms than controls; however, differences did not

reach statistical significance. No differences in static lung function were found between the two groups. Overall, pulmonary abnormalities and/or significant ongoing respiratory symptoms were detected in nearly a third of ALL survivors treated without HSCT. Larger, prospective studies are warranted to evaluate the etiology and clinical significance of these findings.

KEYWORDS
cancer survivors, child, neoplasms, respiratory function tests, respiratory traction infections

1 | INTRODUCTION

Pulmonary dysfunction is one of the most prevalent causes of morbidity and premature mortality in pediatric cancer survivors.[1,2] Long-term follow-up studies have found respiratory-related deaths to be second only to subsequent malignancy, and pulmonary abnormalities have been detected in up to 81% of survivors before the age of 50 years. Of all childhood cancers, acute lymphoblastic leukemia (ALL) is the most common and carries an excellent prognosis with a 10-year survival exceeding 90%.[3] In an era of rising cure rates, late pulmonary effects will become increasingly important for a growing population of survivors.[4]

Historically, research has focused on identifying pulmonary-toxic therapies, such as radiation and hematopoietic stem cell transplant (HSCT), to inform risk-based pulmonary follow-up.[4-6] However, respiratory infections are also a significant cause of pulmonary morbidity during treatment.[7] In particular, recent studies using polymerase chain reaction (PCR) diagnostics have found respiratory viral infections (RVI) cause up to 57% of febrile episodes, and that children undergoing chemotherapy are at risk of more severe RVIs with a prolonged clinical course and higher viral loads.[7-11]

In general population-based studies, respiratory syncytial virus (RSV) and rhinovirus (HRV) bronchiolitis during infancy have been associated with subsequent wheeze and obstructive defects.[12,13] RVIs have also been shown to precipitate late airflow decline[14] and alloimmune lung syndromes[15] in transplant recipients. However, the long-term pulmonary sequelae of these RVIs in children undergoing chemotherapy alone have not been investigated. Currently, the Children's Oncology Group (COG)[16] have not prescribed recommendations for long-term pulmonary function screening for this cohort.

This study aimed to evaluate the long-term pulmonary morbidity of respiratory viral infections during chemotherapy in childhood ALL survivors, as defined by (a) static lung function tests and (b) respiratory symptoms beyond 6 months posttherapy. We hypothesized that children with RVIs during chemotherapy would demonstrate increased respiratory symptoms and poorer lung function, compared to those without RVI during treatment.

2 | MATERIALS AND METHODS

This cross-sectional cohort study of childhood ALL survivors was conducted at two tertiary pediatric hospitals. Lung function assessments were performed to compare respiratory outcomes in children with and without proven RVIs during chemotherapy. Ethical approval was granted by The Sydney Children's Hospitals Network Human Research Ethics Committee (LNR/15/SCHN/309). Written, informed consent was obtained from all caregivers and participants where applicable.

2.1 | Subjects and recruitment

Childhood survivors of primary ALL, aged 7 to 18 years, at least 6 months posttherapy were eligible for this study. Exclusion criteria included: pulmonary-toxic therapy as prescribed by COG long-term follow-up (LTFU) guidelines,[16] including craniospinal irradiation; HSCT; proven bacterial or fungal respiratory infections (microbiologically detected from sputum) during chemotherapy; and developmental or psychosocial preclusions as determined by their oncologist.

Hospital-based oncology databases were reviewed for all patients diagnosed with primary ALL from 1 April 1999 to 1 April 2015 at our two sites. Eligible patients were recruited in an order determined by an online random sequence generator, and classified into the "viral" or "control" group by the presence or absence of retrospectively documented RVIs during chemotherapy respectively. For the purposes of this study, a RVI was defined by (a) microbiologically detected respiratory virus on nasopharyngeal aspirate, sputum, or bronchoalveolar-lavage specimens, associated with (b) clinical acute respiratory illness, defined as any new-onset cough, rhinorrhoea, sore throat, dyspnoea, tachypnoea or abnormal lung examination. All retrospectively documented RVIs satisfying these criteria, in both the inpatient and outpatient setting, were included.

Before June 2011, viral cultures and immunofluorescence were used for the detection of viruses: RSV; HRV (culture only); adenovirus; parainfluenza virus 1, 2, and 3; influenza A and B. Testing for human metapneumovirus (hMPV) became available in September 2008. From June 2011, PCR (MagNa Pure, Roche) was used to test for all aforementioned viruses (See Gene respiratory pathogen panels). From June 2012, PCR testing also included parainfluenza virus 4, bocavirus, and coronaviruses 229E/NL63 and OC43.

2.2 | Study design

All participants underwent an assessment of (a) static lung function testing: forced oscillation technique (FOT), pre and postbronchodilator spirometry, body plethysmography, and diffusion capacity for

carbon monoxide (DLCO) in the order shown, and (b) respiratory symptoms by questionnaire. Participants were clinically well at the time of assessment.

2.3 | Lung function testing

FOT was conducted with a multifrequency composite waveform (5-37 Hz) using the tremoFlo C-100 device (Thorasys, Canada) in accordance with published recommendations.[17,18] A minimum of three technically satisfactory 60 seconds recordings were obtained, as defined by: stable tidal breathing pattern; no signs of cough, leak, glottic closure, or volume drift; and respiratory system resistance at 5 Hz (R5) values within 15%. R5, reactance at 5 Hz (X5), the frequency dependence of resistance (R5-19), resonant frequency (Fres), and reactance area (AX) were reported as the mean value of all satisfactory tests. *Z*-scores for R5, X5, R5-19 and percent predicted values for Fres were calculated using published reference equations,[19] whilst AX was presented as its raw value, as normative data are poorly established within this age range.

Standard spirometry (pre and postbronchodilator), body plethysmography, and single-breath DLCO was then performed (VIASYS Vmax system; Sensormedics, Yorba Linda, CA) as per ATS/ERS standards.[20,21] Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow 25% to 75% (FEF_{25-75}) were expressed as z-scores using Global Lung Initiative reference equations.[22] A positive bronchodilator response was defined by greater than 12% and greater than 200 mL in FEV_1 and/or FVC. Total lung capacity (TLC), residual volume (RV), and DLCO were evaluated as percent predicted values as per published normative data.[23] Due to limitations in blood sampling, DLCO values were uncorrected for hemoglobin. All children with abnormal DLCO values had hemoglobin results within normal limits when tested within the prior 3 months.

In accordance with our laboratory protocol, pulmonary function results were considered abnormal if: FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, R5, X5, R5-19 were beyond 1.64 standard deviations from the reference mean; TLC < 80% predicted, RV > 120% predicted, RV/TLC ratio > 30%; or DLCO < 80% predicted.

2.4 | Respiratory questionnaire

Respiratory symptoms were assessed by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[24] and the Liverpool Respiratory Symptom Questionnaire (LRSQ).[25]

The ISAAC questionnaire evaluates the presence of atopic diseases including asthma, eczema, hay fever and their associated symptoms.

The LRSQ consists of 32 questions across 8 domains, assessing respiratory symptoms and their impact on the child and family over the last 3 months (Table 1). Each item was scored on a five-point Likert scale from "not at all" (0) to "everyday" (4) and then totaled for a domain score and overall score. As previously recommended, the snoring question in the "night-time" domain was excluded.[25] The prevalence of day, night, and exercise symptoms including wheeze, cough, and dyspnoea were also extracted. Responses "not at all" or "few days" were classified as "infrequent"; and responses "some days", "most days", or "everyday" were classified as "frequent." These symptoms were specifically analyzed, as they were considered to be clinically significant and have been noted in the COG-LTFU guidelines.[16]

2.5 | Sample size

The primary outcome measure for this study was the total respiratory system resistance at 5 Hz, as determined by FOT. This was chosen for its sensitivity to peripheral airway function.[26] Based on a previous asthma study,[27] a difference of 0.99 cm H_2O/L/s was considered clinically meaningful. Assuming a standard deviation of 1.25, 26 subjects per group (52 total) were required for a power of 80% at 5% statistical significance. During the testing period, recruitment between the two groups was unequal. Power calculations demonstrated that for a patient ratio of 1.3:1, 30 viral patients and 23 controls were needed.

2.6 | Data analysis

Primary comparisons between the viral and control groups were made using the student *t* test for continuous variables, and Fisher's exact test for categorical variables. To adjust for potential confounders, multiple regression models were built for each lung function and symptom outcome using the forwards sequential method. Previous RVI was retained in the model as the variable of interest. Covariates including sex, prematurity, maternal smoking, site, age at diagnosis, years posttherapy, ALL type, ALL risk, and ALL protocol were tested by Pearson's correlation, *t* test, or Fishe's exact tests and then added in order of univariate significance. Variables

TABLE 1 Liverpool respiratory symptom questionnaire domains

Domain	Items assessed
1. Daytime symptoms	
2. Night-time symptoms	Wheeze
3. Symptoms with colds	Cough
4. Interval symptoms (without colds)	Rattly chest
5. Activity or exercise-related symptoms	Shortness of breath
6. Other symptoms	Noisy breathing not from the chest, noisy breathing from the throat, fast breathing
7. Effects on child	Eating, activity, sleep, fatigue
8. Effects on family	Family activities, life adjustment, sleep, worry

Note: The Liverpool Respiratory Symptom Questionnaire consists of eight domains which assess respiratory symptoms and their impact on the child/family over the last three months. The frequency of each item is indicated on a five-point Likert scale and assigned a corresponding score, from "not at all" (0) to "everyday" (4). Item scores are totalled for a domain score and overall score.

were retained if their P value was < .10 without inflating standard error greater than 10%. Due to correlation between "years posttherapy" and "age at testing" (P < .001), and "age at testing" being already adjusted for by lung function endpoints expressed as z-scores, only "years posttherapy" was tested for a parsimonious model.

Statistics were performed using SPSS version 23.0 (IBM) with significance assigned when P < .05.

3 | RESULTS

3.1 | Subjects

Between 1999 and 2015, 355 children treated for ALL across our two institutions were eligible for this study. Of the 116 patients contacted, 54 (47%) were enrolled (Figure 1). Demographic and clinical characteristics were not different between those patients who consented and those who declined to participate (Table S1).

Baseline characteristics of our study population (N = 54) are displayed in Table 2. The viral (n = 31) and control (n = 23) group had no significant differences in demographic or clinical characteristics. Children were of a median (range) age of 11.2 (7.2-18.1) years when evaluated within this study, originally diagnosed with ALL at 4.5 (1.1-13.5) years of age, and evaluated at 4.9 (0.5-13) years posttherapy.

All patients were treated on 2-year regimens with Berlin-Franklin-Munster based protocols: BFM-95 (before 2002),[28] ANZCHOG Study 8 (2002-2011),[29] or AEIOP-BFM ALL 2009 study (after 2011). Chemotherapy regimen included methotrexate, cyclophosphamide, and anthracycline. Cumulative exposure to steroids was 3413 mg/m^2 prednisone (1 mg dexamethasone = 6.67 mg prednisone), and high-risk patients received additional reconsolidation therapy with dexamethasone (equivalent to 2934 mg/m^2 prednisone) and high-dose methotrexate (15 g/m^2).

3.2 | Respiratory viruses during chemotherapy

Patients in the viral group (n = 31) had a median of 3 (range 1-7) proven RVIs during chemotherapy. Ten (32%) patients had a single infection, 5 (16%) had two, 8 (26%) had three, 6 (19%) had four, 1 (3%) had six, and 1 (3%) had seven. Of the total 81 RVI episodes documented, 65 (80%) involved hospital admission and 16 (20%) were detected in the outpatient setting. All "viral" patients had been admitted for at least one RVI, with a mean hospital stay of 4.3 days per episode. The most commonly detected virus was HRV (27 of 81), followed by RSV (25 of 81), PIV 3 (9 of 81) and influenza A (9 of 81), parainfluenza type 3 (7 of 81), influenza B (6 of 81), hMPV (6 of 81), adenovirus (3 of 81), parainfluenza type 1 (3 of 81), coronavirus 229 (2 of 81) parainfluenza type 2 (1 of 81), and coronavirus OC43 (1 of 81). Twenty-one (68%) children had ever isolated RSV, 16 (32%) ever isolated HRV, and 6 (11%) ever isolated hMPV. Fourteen (45%) children had coinfections with greater than 2 viruses. The mean time between the last viral episode and lung function testing was 6.1 (range, 1.9-14) years.

3.3 | Lung function testing

Acceptable and repeatable FOT, spirometry, body plethysmography and DLCO data were obtained in 48 (88%), 51 (94%), 48 (88%), and

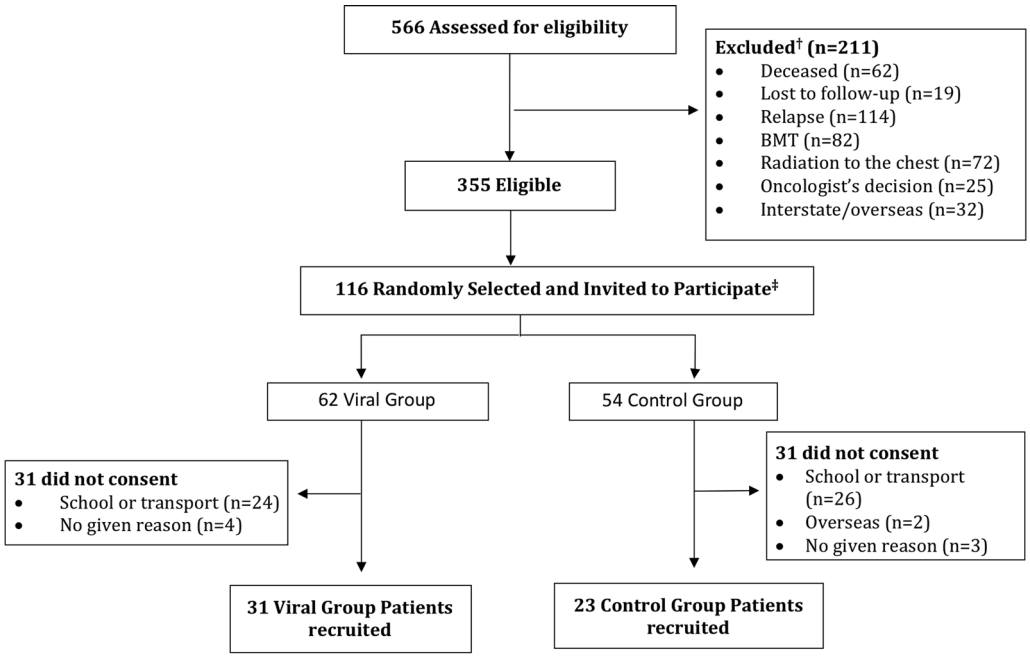

FIGURE 1 CONSORT flow diagram of recruitment. †Patients may have been excluded due to several criteria. ‡Note: After sufficient patients were recruited for the viral group, patients continued to be randomly selected until adequate controls were recruited. In this period, 13 additional "viral" patients were randomly selected, but not invited to participate

TABLE 2 Patient characteristics in viral and control groups

	Viral (n = 31)	Control (n = 23)	All (N = 54)
Male, n (%)	17 (55%)	17 (74%)	34 (63%)
Age, y	11.2 (7.2-18.1)	12.7 (7.5-18.1)	11.2 (7.2-18.1)
Height, cm	145.3 ± 17.2	154.6 ± 18.0	149.3 ± 18.0
Weight, kg	45.9 ± 16.2	50.6 ± 16.4	47.9 ± 16.3
Premature,[a] n (%)	2 (6%)	4 (13%)	6 (11%)
Years posttherapy	5.2 (0.5-13)	4.3 (1.3-10.5)	4.9 (0.5-13)
Age at diagnosis, y	3.3 (1.1-11.4)	4.6 (1.9-13.5)	4.4 (1.1-13.5)
ALL type, n (%)			
Pre-B cell	31 (100%)	19 (83%)	50 (93%)
T-cell	0 (0%)	4 (17%)	4 (7%)
ALL risk, n (%)			
High	2 (6%)	1 (4%)	3 (6%)
Medium	14 (45%)	11 (48%)	25 (46%)
Standard	15 (48%)	11 (48%)	26 (48%)
ALL protocol, n (%)			
Study 9	5 (16%)	5 (22%)	10 (19%)
Study 8	23 (74%)	18 (78%)	41 (76%)
BFM95	3 (10%)	0 (0%)	3 (5%)
Duration of treatment protocol, y	2	2	2

Note: No statistically significant differences were found between the viral and control groups for any parameter. Data are presented as mean ± SD or median (range), unless otherwise stated.
[a]Premature: defined as less than 37 weeks gestation.

TABLE 3 Lung function results in viral and control groups

	Viral (n = 31)	Controls (n = 23)	*P* value
FOT, n	27	21	
R5, cm H_2O/L/s	4.99 (1.60)	4.80 (1.92)	0.72
z-score	−0.27 (0.41)	0.01 (0.48)	0.27[a]
X5, cm H_2O/L/s	−2.02 (0.81)	−1.78 (0.94)	0.33
z-score	−0.25 (0.95)	−0.45 (1.31)	0.37[b]
R5-19, cm H_2O/L/s	0.86 (0.80)	0.79 (1.13)	0.80
z-score	0.08 (0.20)	0.10 (0.31)	0.77
Fres, Hz	19.2 (5.4)	16.6 (5.3)	0.11
% Predicted	128.5 (29.9)	121.7 (29.0)	0.43
AX, cm H_2O/L	15.9 (12.1)	12.3 (11.0)	0.29
Spirometry, n	29	22	
FEV_1% predicted	105.0 (12.2)	102.3 (10.4)	0.41
z-score	0.43 (1.05)	0.20 (0.89)	0.41
FVC % predicted	106.7 (12.6)	104.8 (12.8)	0.61
z-score	0.54 (1.05)	0.41 (1.08)	0.65[c]
FEV_1/FVC ratio, %	86.3 (5.1)	84.8 (6.6)	0.39
z-score	−0.23 (0.70)	−0.30 (1.07)	0.81[d]
FEF25-75 z-score	0.09 (1.02)	−0.26 (0.97)	0.23
Body plethysmography, n	27	21	
TLC % predicted	101.6 (13.1)	102.6 (14.8)	0.76[e]
RV % predicted	85.1 (34.5)	83.8 (25.6)	0.89
RV/TLC ratio, %	18.2 (5.3)	17.1 (4.2)	0.45
DLCO, n	27	23	
% Predicted	91.0 (12.9)	85.3 (16.1)	0.99[f]

Note: Data are presented as mean (SD), unless otherwise stated.
Abbreviations: AX, reactance area; FEV_1, forced expiratory volume in 1 second; Fres, resonant frequency; FVC, forced vital capacity; R5-10, frequency dependence of resistance; Rrs 5 Hz, respiratory system resistance at 5 Hz; RV, residual volume; TLC, total lung capacity; Xrs 5 Hz, reactance at 5 Hz.
[a]*P* values were adjusted for site.
[b]*P* values were adjusted for sex and prematurity.
[c]*P* values were adjusted for age at diagnosis for less than 5 years.
[d]*P* values were adjusted for ALL type.
[e]*P* values were adjusted for sex.
[f]*P* values were adjusted for sex in multivariable models.

50 (93%) subjects, respectively (Table 3). No significant differences in any lung function parameter were observed between the viral and control groups.

For all lung function tests, mean results were within normal limits for both the viral and control groups. Individually, 14 (26%) patients (6 viral; 8 control) had one or more abnormal lung function parameter. The most common abnormality was reduced DLCO, with 8 mild (60%-79% predicted) and 3 moderate (40%-59% predicted) degrees of impairment. Seven subjects (13%) had decreased reactance (X5; − 4.43 to 1.92 z-score), with none demonstrating abnormal respiratory resistance (R5, R5-19). Eleven (20%) children (5 viral; 6 control) had Fres values greater than 150% predicted. Two patients (1 viral; 1 control) had decreased FEV_1 and FEF25-75 consistent with obstructive disease, and one subject had a positive bronchodilator response. Three (5%) patients had mild reductions in TLC, and two (4%) had an increased RV/TLC ratio. Of the 14 children with abnormalities, 6 reported respiratory symptoms and 8 were asymptomatic. A further three children with normal lung function testing demonstrated significant respiratory symptoms warranting follow-up as determined by a respiratory physician. The demographic, clinical, and lung function profiles of these 17 (31%) patients with pulmonary function and/or symptomatic abnormalities are detailed in Table S2.

3.4 | Respiratory symptoms and quality of life

The prevalence of wheeze, cough, and dyspnea in each group are shown in Table 4. No differences reached statistical significance. The largest effect size found was that the viral group demonstrated a threefold increase in odds of current wheeze (45% vs 22%; odds ratio [OR], 3.0; *P* = .09), and nocturnal cough (45% vs 26%; OR, 2.7; *P* = .11). As underlying asthma could be an intervening variable, subgroup analysis in the 48 non-asthmatic patients (27 viral, 21 control) was performed, revealing similar results: wheeze OR, 3.5 (*P* = .10), and nocturnal cough OR, 2.9 (*P* = .13).

TABLE 4 Respiratory symptoms in viral and control groups

	Viral (n = 31)	Control (n = 23)	OR (95% CI)	*P* value
Wheeze, current	45%	22%	3.0 (0.9, 10.0)[a]	.09
Day time, frequent				
Cough	39%	30%	1.4 (0.5, 4.5)[a]	.57
Dyspnea	13%	13%	1.0 (0.2, 4.9)[a]	1.00
Night time, frequent				
Cough	45%	26%	2.7 (0.8, 9.5)[b]	.11
Dyspnea	10%	9%	1.1 (0.2, 7.3)[a]	1.00
Exercise-induced, frequent				
Wheeze	13%	9%	1.6 (0.3, 9.3)[a]	1.00
Cough	32%	22%	1.7 (0.5, 6.0)[a]	.54
Dyspnea	23%	13%	1.9 (0.4, 8.5)[a]	.49

[a]In the final multivariate model built using the forwards sequential method, no covariates were retained. Thus, this OR is unadjusted.
[b]Adjusted for years posttherapy greater than 5 years.

The viral group totaled a slightly higher overall LRSQ score (17.7 vs 14.5, $P = .51$), with increased scores across every symptom and quality of life domain except "symptoms during colds" which were equivalent to the control group (Figure 2). Statistical significance was only reached in the "other symptoms" domain, which assessed "noisy breathing not from the chest," "noisy breathing from the throat" and "fast breathing".

ISAAC questionnaire results demonstrated that the prevalence of atopic disease was similar between the viral and control groups: asthma 4 of 31 vs 2 of 23 ($P = 1.00$); allergic rhinitis 4 of 31 vs 2 of 23 ($P = 1.00$); eczema 6 of 31 vs 2 of 23 ($P = .44$).

4 | DISCUSSION

To our knowledge, this is the first study to investigate the long-term pulmonary morbidity of respiratory viruses during chemotherapy in childhood cancer survivors. Compared to controls, children with RVIs during chemotherapy had more ongoing respiratory symptoms particularly wheeze and cough, although these results did not reach statistical significance. No differences in static lung function were found between the two groups. Overall, pulmonary abnormalities were detected in nearly a third of ALL survivors when evaluated up to 13 years posttherapy. Of note, these patients were treated without pulmonary-toxic therapies recognized by the COG-LTFU guidelines and thus, do not receive routine lung function screening.

Our results suggest that children with proven RVIs during chemotherapy may be at risk of developing more long-term respiratory symptoms. A threefold increase in odds of wheeze and nocturnal cough was observed, and the viral group reported a higher prevalence in nearly all other symptoms and LRSQ scores. These results however were not statistically significant. As our study was not powered for symptom outcomes, particularly for wheeze (OR, 2.7, $P = .09$) and cough (OR, 2.7, $P = .11$), the moderate effect sizes were considered a type II error and suggest larger studies are warranted in future. These findings are similarly documented following infant RSV and HRV bronchiolitis, where RVIs have been associated with subsequent asthma and atopy.[12,13] While the pathogenesis of early-life respiratory viruses remains unknown, some studies have implicated excess type 2 immunity and aberrant lung remodeling.[30,31] The complexity of this however, is further highlighted by studies which suggest that inherent susceptibility to any type of respiratory episode, including bacterial infections, is more important than viral triggers.[32] In our study, only one child had a positive bronchodilator response and atopy was low in both the viral and control groups. This suggests a different phenotype of disease in our cohort, who were older but more severely immunosuppressed. We hypothesize cancer survivors may have suffered direct tissue damage or neuroimmune modifications of respiratory mucosa following the RVI; however, further study is required to examine the role of other pathogens such as bacteria. As with infant bronchiolitis, there also remains the question of cause and effect: whether RVIs during chemotherapy predispose respiratory morbidity, or if children with pre-existing defects are more susceptible to RVIs.

Static lung function testing found no differences between the viral and control groups. In isolation, this is an important negative finding that supports conservative management of RVIs during cancer therapy and suggests viral infections do not confer long-term airflow defects. It is possible that immunosuppression and a failure to mount inflammatory responses were protective against scarring and lung damage.[33,34] This is however, incongruent with a 12-year retrospective study reporting late airflow decline associated with RVIs in HSCT recipients, independent of alloimmune lung disease.[14] Here, prolonged viral-shedding and subsequent airway inflammation were proposed to be the mechanisms of injury. These findings suggest that the pathogenesis of RVIs in immunosuppressed cohorts is unique and remains poorly understood.

In our patients with RVIs, there may be several explanations for the preservation of lung function despite an increase in respiratory symptoms. Firstly, the reliability of self-reported symptoms may be questioned; however a difference in the same patient-reported outcomes was observed using validated questionnaires (LRSQ and ISAAC) between our viral and control group. Alternatively, our lung function tests only assessed static physiology and may not have stressed the pulmonary system to detect functional abnormalities as possible with cardiopulmonary exercise testing. Elevated Fres values in this cohort, which have been previously associated with increased capacitance and distal airway pathology, further suggest that multiple breath washout may be needed to better characterize peripheral ventilation inhomogeneity.[35]

This study also provided a cross-sectional evaluation of lung function in a homogeneous cohort of ALL survivors, treated on contemporary protocols without HSCT or chest radiation. Pulmonary abnormalities were detected in 31% of individuals, of whom half were asymptomatic. The most common defects were reduced DLCO and X5, a parameter which reflects lung stiffness[35] and may suggest a

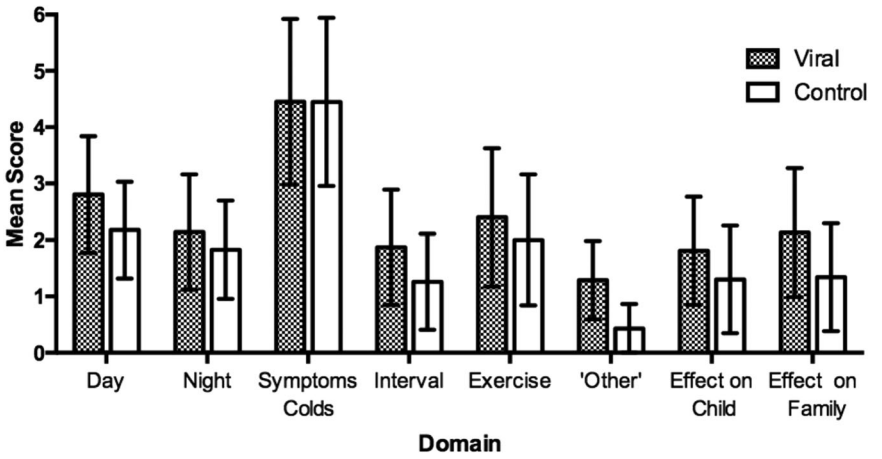

FIGURE 2 Liverpool respiratory symptom questionnaire score results. Comparison of the frequency of respiratory symptoms and their impact on the child and family between the viral and control groups

picture of fibrosis. Although imaging was not a part of our formal study protocol due to ethical considerations; of the 11 children with abnormal DLCO values, four received follow-up HRCTs which all demonstrated diffuse mild to moderate gas trapping and were suggestive of smaller airways disease. While it is difficult to draw conclusions from this limited sample, these findings contribute to the justification for further investigation.

Five studies have previously reported on the pulmonary function of childhood leukemia survivors, none of which analyzed the impact of respiratory infections.[36-40] These found restrictive lung defects and diffusion impairment in up to 65% of patients, most of whom were also asymptomatic. This literature predates 2000, and mostly investigated patients treated with recognized pulmonary-toxic therapies (HSCT or chest radiation). A recent study of leukemia and lymphoma survivors has been conducted in an Egyptian institution with routine pulmonary function, impulse oscillometry, and CT scans.[41] Although diffusion capacity was not assessed, subclinical abnormalities were detected in 52% of the 25 ALL survivors. In contrast to our study, Tantawy et al[41] reported elevated R5 in 25% of their cohort which may be due to their heterogeneous patient cohort and a different chemotherapy regimen. There is a paucity of literature describing the long-term lung function in contemporary cancer survivors treated without established pulmonary-toxic therapies. Although it was not the primary aim of this study, our finding of abnormality in nearly a third of ALL survivors suggests research is warranted to investigate unrecognized aetiologies and to inform the need for respiratory surveillance and future LTFU guidelines.

To date, this is one of the largest cross-sectional lung function studies in leukemia survivors, and the only report to examine the late morbidity of respiratory viruses. All lung function data were cross-sectionally collected and included objective pulmonary function measures and patient-related outcomes. Our patients were a homogenous cohort of primary ALL survivors, treated on contemporary chemotherapy protocols. Exclusion of children exposed to known pulmonary-toxic therapies and proven bacterial/fungal respiratory infections enabled respiratory viruses to be examined with minimal confounders.

However, this retrospective study also had a number of limitations. With our relatively small sample size, results did not reach statistical significance and thus these data should only be considered hypothesis-generating. While sufficient patients were recruited to test R5, power calculations were based on an asthma study, and this study was not powered to evaluate specific risk factors or viruses and their subspecies. To preserve a parsimonious model, only covariates with a univariate $P > 0.1$ could be retained in the multivariate regression analysis. Thus some clinically important factors, such as "age at diagnosis" were not retained as they were weak predictors in our small sample size, and require larger studies to power statistical models that can meaningfully test the impact of covariates. As this was a retrospective study, we relied on past medical records of microbiological testing on sputum samples collected from patients at the time of therapy. These data were used to classify the "viral" and "control" groups, and exclude children with proven respiratory bacterial and fungal infections, although sputum samples would not have been routinely tested on all patients. Incomplete medical records also precluded characterization of RVIs by severity, and as upper or lower tract infections. Furthermore, the microbiological methods used to isolate respiratory viruses were variable, where cell culture and immunofluorescence had limitations in the diagnosis of specific viruses such as HRV and HMPV as compared to newer molecular techniques. Thus, our viral cohort may only represent children with severe infections warranting investigation, or only those with viruses detectable by the microbiological method available at time of testing. As these children did not receive pulmonary assessments prior or during therapy, we also lack longitudinal data to evaluate trends in pulmonary function or the chronology of respiratory symptoms with respect to therapy.

Future investigation into the long-term sequelae of respiratory viruses requires prospective, longitudinal studies from diagnosis to post-therapy with the standardized use of active PCR-based viral surveillance. More comprehensive lung function testing, such as with cardiopulmonary exercise testing and multiple breath washout, may help further characterize and underlying clinical and subclinical lung function deficits. Understanding the prevalence, etiology, and trajectory of pulmonary dysfunction into adulthood will be important to inform future LTFU guidelines and survivor care.

5 | CONCLUSION

Our study found children with respiratory viral infections during chemotherapy developed more long-term respiratory symptoms than controls, however these differences were not statistically significant. No differences in static lung function outcomes were found between the two groups. Overall, pulmonary abnormalities and/or significant ongoing respiratory symptoms were detected in nearly a third of our ALL survivors treated with non-myeloablative chemotherapy, when evaluated up to 13 years posttreatment. These findings warrant future prospective studies into the etiology, clinical significance, and therapeutic options for these respiratory complications. Long-term respiratory follow-up should be considered in ALL survivors to facilitate early detection and management of late pulmonary effects.

ACKNOWLEDGMENTS

We are sincerely grateful to the patients and families who participated in this study. We also wish to thank the oncologists of the Sydney Children's Hospitals Network for their valuable support, and Professor Jenny Peat for her statistical advice.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Beryl Lin http://orcid.org/0000-0002-2299-9770

REFERENCES

1. Hudson MM, Ness KK, Gurney JG, et al . Clinical ascertainment of health outcomes among adults treated for childhood cancer. *JAMA*. 2013;309(22):2371-2381.
2. Mertens AC, Liu Q, Neglia JP, et al. Cause-specific late mortality among 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *J Natl Cancer Inst*. 2008;100(19):1368-1379.
3. Pui C, Pei D, Campana D, et al. A revised definition for cure of childhood acute lymphoblastic leukemia. *Leukemia*. 2014;28(12):2336-2343.
4. Dietz AC, Chen Y, Ness KK, et al. Risk and impact of pulmonary complications in survivors of childhood cancer: a report from the Childhood Cancer Survivor Study (CCSS). 2014. p 10022.
5. Goss CH. With every upside, there is a downside: chest radiation and survivors of childhood cancers. Am Thoracic Soc; 2016;13(9): 1448–1449.
6. Record E, Williamson R, Wasilewski-Masker K, Mertens AC, Meacham LR, Popler J. Analysis of risk factors for abnormal pulmonary function in pediatric cancer survivors. *Pediatr Blood Cancer*. 2016; 63(7):1264-1271.
7. Hakim H, Dallas R, Zhou Y, et al. Acute respiratory infections in children and adolescents with acute lymphoblastic leukemia. *Cancer*. 2016;122(5):798-805.
8. Christensen MS, Nielsen LP, Hasle H. Few but severe viral infections in children with cancer: a prospective RT-PCR and PCR-based 12-month study. *Pediatr Blood Cancer*. 2005;45(7):945-951.
9. Lindblom A, Bhadri V, Soderhall S, and . others. Respiratory viruses, a common microbiological finding in neutropenic children with fever. *J Clin Virol*. 2010;47(3):234-237.
10. Koskenvuo M, Mottonen M, Rahiala J, et al. Respiratory viral infections in children with leukemia. *Pediatr Infect Dis J*. 2008;27(11):974-980.
11. Srinivasan A, Gu Z, Smith T, et al. Prospective detection of respiratory pathogens in symptomatic children with cancer. *Pediatr Infect Dis J*. 2013;32(3):e99-e104.
12. Perez-Yarza EG, Moreno A, Lazaro P, Mejias A, Ramilo O. The association between respiratory syncytial virus infection and the development of childhood asthma: a systematic review of the literature. *Pediatr Infect Dis J*. 2007;26(8):733-739.
13. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008;178(7):667-672.
14. Erard V, Chien JW, Kim HW, et al. Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. *J Infect Dis*. 2006;193(12):1619-1625.
15. Versluys AB, Rossen JW, van Ewijk B, Schuurman R, Bierings MB, Boelens JJ. Strong association between respiratory viral infection early after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic alloimmune lung syndromes. *Biol Blood Marrow Transplant*. 2010;16(6):782-791.
16. Children's Oncology Group. Long-term follow-up guidelines for survivors of childhood, adolescent, and young adult cancers. 2013. www.survivorshipguidelines.org/pdf/LTFUResourceGuide.pdf
17. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J*. 2003;22(6):1026-1041.
18. Robinson PD, Turner M, Brown NJ, et al. Procedures to improve the repeatability of forced oscillation measurements in school-aged children. *Respir Physiol Neurobiol*. 2011;177(2):199-206.
19. Nowowiejska B, Tomalak W, Radliński J, Siergiejko G, Latawiec W, Kaczmarski M. Transient reference values for impulse oscillometry for children aged 3–18 years. *Pediatr Pulmonol*. 2008;43(12):1193-1197.
20. Clausen J, Coates A, Quanjer P. Measurement of lung volumes in humans: review and recommendations from an ATS/ERS workshop. *Eur Respir J*. 1997;10(6):1205-1206.
21. Crapo R, Hankinson J, Irvin C, MacIntyre N, Zoter K, Rise R. Single-breath carbon monoxide diffusing capacity (Transfer factor). Recommendations for a standard technique-1995 Update. *Am J Respir Crit Care Med*. 1995;152:2185-2198.
22. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J*. 2012;40(6):1324-1343.
23. Polgar G, Varuni P. *Pulmonary Function Testing in Children: Techniques and Standards*. Philadelphia: Saunders Limited. 1971.
24. Asher M, Keil U, Anderson H, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8(3):483-491.
25. Trinick R, Darracott C, Southern K, McNamara P. Validation of the Liverpool Respiratory Symptom Questionnaire (LRSQ) in well children with cystic fibrosis (CF). *J Cyst Fibrosis*. 2010;9:S57.
26. Larsen GL, Morgan W, Heldt GP, et al. Impulse oscillometry versus spirometry in a long-term study of controller therapy for pediatric asthma. *J Allergy Clin Immunol*. 2009;123(4):861-867.
27. Robinson PD, Brown NJ, Turner M, Van Asperen P, Selvadurai H, King GG. Increased day-to-day variability of forced oscillatory resistance in poorly controlled or persistent pediatric asthma. *Chest J*. 2014;146(4):974-981.
28. Möricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. *Blood*. 2008;111(9):4477-4489.

29. Marshall G, Dalla Pozza L, Sutton R, et al. High-risk childhood acute lymphoblastic leukemia in first remission treated with novel intensive chemotherapy and allogeneic transplantation. *Leukemia*. 2013;27(7):1497-1503.
30. Legg JP, Hussain IR, Warner JA, Johnston SL, Warner JO. Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. *Am J Respir Crit Care Med*. 2003;168(6):633-639.
31. Caliskan M, Bochkov YA, Kreiner-Moller E, and . others. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med*. 2013;368(15):1398-1407.
32. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol*. 2015;136(1):81-86.
33. Lehrnbecher T, Foster C, Vázquez N, Mackall CL, Chanock SJ. Therapy-induced alterations in host defense in children receiving therapy for cancer. *J Pediatr Hematol Oncol*. 1997;19(5):399-417.
34. Hersh EM, Carbone PP, Wong VG, Freireich EJ. Inhibition of the primary immune response in man by anti-metabolites. *Cancer Res*. 1965;25(7, pt 1):997-1001.
35. Smith H, Reinhold P, Goldman M. Forced oscillation technique and impulse oscillometry. *Eur Resp Monograph*. 2005;31:72.
36. Jenney ME, Faragher EB, Jones PHM, Woodcock A. Lung function and exercise capacity in survivors of childhood leukaemia. *Med Pediatr Oncol*. 1995;24(4):222-230.
37. Nysom K, Holm K, Olsen J, Hertz H, Hesse B. Pulmonary function after treatment for acute lymphoblastic leukaemia in childhood. *Br J Cancer*. 1998;78(1):21-27.
38. Fulgoni P, Zoia MC, Corsico A, et al. Lung function in survivors of childhood acute lymphoblastic leukemia. *Chest J*. 1999;116(5):1163-1167.
39. Miller RW, Fusner JE, Fink RJ, et al. Pulmonary function abnormalities in long-term survivors of childhood cancer. *Med Pediatr Oncol*. 1986;14(4):202-207.
40. Shaw N, Tweeddale P, Eden O. Pulmonary function in childhood leukaemia survivors. *Med Pediatr Oncol*. 1989;17(2):149-154.
41. Tantawy AAG, Elbarbary N, Ahmed A, Mohamed NA, Ezz-Elarab S. Pulmonary complications in survivors of childhood hematological malignancies: single-center experience. *Pediatr Hematol Oncol*. 2011;28(5):403-417.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Lin B, Kennedy B, McBride J, et al. Long-term morbidity of respiratory viral infections during chemotherapy in children with leukaemia. *Pediatric Pulmonology*. 2019;54:1821-1829. https://doi.org/10.1002/ppul.24456