MAJOR ARTICLE

Parainfluenza Virus Lower Respiratory Tract Disease After Hematopoietic Cell Transplant: Viral Detection in the Lung Predicts Outcome

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Background. Parainfluenza virus (PIV) commonly infects patients following hematopoietic cell transplantation (HCT), frequently causing lower respiratory tract disease (LRTD). The definition of LRTD significantly differs among studies evaluating the impact of PIV after HCT.

Methods. We retrospectively evaluated 544 HCT recipients with laboratory-confirmed PIV and classified LRTD into 3 groups: possible (PIV detection in upper respiratory tract with new pulmonary infiltrates with/without LRTD symptoms), probable (PIV detection in lung with LRTD symptoms without new pulmonary infiltrates), and proven (PIV detection in lung with new pulmonary infiltrates with/without LRTD symptoms).

Results. Probabilities of 90-day survival after LRTD were 87%, 58%, and 45% in possible, probable, and proven cases, respectively. Patients with probable and proven LRTD had significantly worse survival than those with upper respiratory tract infection (probable: hazard ratio [HR], 5.87 [$P < .001$]; proven: HR, 9.23 [$P < .001$]), whereas possible LRTD did not (HR, 1.49 [$P = .27$]). Among proven/probable cases, oxygen requirement at diagnosis, low monocyte counts, and high-dose steroid use (>2 mg/kg/day) were associated with high mortality in multivariable analysis.

Conclusions. PIV LRTD with viral detection in lungs (proven/probable LRTD) was associated with worse outcomes than was PIV LRTD with viral detection in upper respiratory samples alone (possible LRTD). This new classification should impact clinical trial design and permit comparability of results among centers.

Keywords. parainfluenza virus; lower respiratory tract disease; hematopoietic cell transplant; classification.

Community-acquired respiratory viruses cause lower respiratory tract disease (LRTD) after hematopoietic cell transplantation (HCT), which is associated with high morbidity and mortality [1, 2]. The definition of LRTD associated with respiratory viruses differs in various studies of outcomes and risk factors for mortality following LRTD [3–8]. The strictest definition of LRTD requires LRTD symptoms, abnormal chest radiography, and viral detection in lower respiratory samples, whereas a less stringent definition often consists of LRTD symptoms and a positive nasopharyngeal sample. This spectrum of definitions for LRTD likely includes a broad range of disease stages, resulting in difficulty interpreting and comparing published study results.

Parainfluenza virus (PIV) is a respiratory virus that frequently infects HCT recipients [9–11]. In previous studies, the mortality among patients with LRTD ranged from 13% to 63% [3–6, 11, 12]. Although factors such as copathogens, mechanical ventilation, underlying disease, and steroid use have been reported as risk factors for mortality [4–6], it is not known whether these factors independently predict poor outcomes in HCT recipients.

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We hypothesized that the differences in definitions of LRTD used in prior studies are a major determinant of the observed variability in outcome. The purpose of this study was to classify PIV LRTD according to the site of virologic detection and clinical manifestations, and to compare clinical outcomes among the groups. Moreover, we examined various risk factors for overall mortality and death due to respiratory failure.

METHODS

Study Design

This retrospective cohort study includes patients who first received transplant between December 1990 and December 2011 at the Fred Hutchinson Cancer Research Center (FHCRC) and had documented PIV infection after HCT that was virologically diagnosed at the University of Washington Virology Laboratories (a subset of patients were included in a previously published report [4]). Only an individual's first episode of PIV infection was analyzed. Patients' demographic data and transplant information closest to the PIV infection were retrieved from the FHCRC database, and other data related to the clinical course of PIV infections were collected by medical record review. The study was approved by the Institutional Review Board at FHCRC.

Definitions

PIV detection was performed by conventional culture, direct fluorescent antibody tests, and/or reverse transcription polymerase chain reaction (RT-PCR) assay in respiratory samples. PIV upper respiratory tract infection (URTI) was defined as PIV detection in a nasopharyngeal or sputum sample, with URTI symptoms but no new pulmonary infiltrates. LRTD was divided into 3 groups: possible, probable, and proven. Possible LRTD was defined as PIV detection in a nasopharyngeal or sputum sample with new pulmonary infiltrates (but without confirmation of PIV in the lower respiratory tract) with or without LRTD signs or symptoms (eg, cough, wheezing, rales, tachypnea, shortness of breath, dyspnea, or hypoxia). Probable LRTD was defined as PIV detection in a bronchoalveolar lavage (BAL) or lung biopsy sample with LRTD symptoms, with or without pulmonary function decline, and without new pulmonary infiltrates. The definition of proven LRTD was PIV detection in a BAL or biopsy sample with new pulmonary infiltrates with or without LRTD symptoms.

Viral load was determined by quantitative RT-PCR using stored frozen repository samples [13]. Peak steroid dose was recorded from the period within 2 weeks before PIV infection in patients with URTI. In PIV LRTD cases, peak steroid doses were recorded from within 2 weeks before and after LRTD diagnosis, respectively, and exact steroid dose at 1 month after diagnosis was also collected. Death caused by respiratory failure was defined as any death caused exclusively or predominantly by respiratory failure [14].

Statistical Analysis

Patients' demographic characteristics were summarized and compared among disease categories using χ^2 or Fisher exact test for categorical variables, and Wilcoxon rank-sum test for continuous variables (as appropriate). Wilcoxon rank-sum or Kruskal-Wallis test was utilized for comparisons of continuous variables. The probability of overall survival was estimated using the Kaplan-Meier method. The probability of mortality caused by respiratory failure and incidence of mechanical ventilation were estimated by cumulative incidence curves, treating death due to other causes as a competing risk event. The log-rank test was used to compare univariable hazards of time-to-event outcomes between disease categories. Cox proportional hazards models were used to evaluate unadjusted and adjusted hazard ratios (HRs) for mortality or respiratory mortality, and associated 95% confidence intervals (CIs) were reported. Variables with $P \leq .1$ in the univariable models were candidates for multivariable models. Two-sided P values <.05 were considered statistically significant.

RESULTS

Patient Characteristics

A total of 544 patients had PIV infection following HCT; 345 (63%) and 199 (37%) had URTI and LRTD disease, respectively. LRTD classification of patients included 78 (39%) possible, 19 (10%) probable, and 102 (51%) proven cases of LRTD. Characteristics of each PIV infection group are shown in Table 1. The median time to PIV infection and PIV LRTD after HCT was 71.5 days (range, 0–3140 days) and 78 days (range, 3–3140), respectively.

Mortality in Each Disease Category

The probabilities of overall survival and mortality from respiratory failure at 90 days following PIV diagnosis in patients with URTI or LRTD are shown in Figure 1*A* and 1*B* (overall survival: 91% in URTI, 62% in LRTD; mortality from respiratory failure: 2.5% in URTI, 28% in LRTD) ($P < .001$ for both comparisons). Next, we analyzed the probabilities of overall survival and death caused by respiratory failure among LRTD cases, comparing the 3 disease categories: possible, probable, and proven (Figure 1*C* and 1*D*). The probabilities of 90-day survival after PIV LRTD were 87%, 58%, and 45% in possible, probable and proven cases, respectively ($P < .001$; Figure 1*C*). The presence of LRTD symptoms in possible or proven cases resulted in worse survival than when no LRTD symptoms were present (Supplementary Figure 1*A* and 1*B*). Mortality rates in each group are shown in Table 2.

Table 1. Characteristics of All Patients With Parainfluenza Virus Infection

Characteristic	Total (N = 544)	URTI (n = 345)	LRTD			*P* Value
			Possible (n = 78)	Probable (n = 19)	Proven (n = 102)	
Sex						.11
Male	321 (59)	201 (58)	40 (51)	15 (79)	65 (64)	
Female	223 (41)	144 (42)	38 (49)	4 (21)	37 (36)	
Age at transplant, y						.14
≤20	105 (19)	71 (20)	19 (24)	3 (16)	12 (12)	
21–60	365 (67)	227 (66)	45 (58)	15 (79)	78 (77)	
>60	74 (14)	47 (14)	14 (18)	1 (5)	12 (12)	
Transplant year[a]						<.001
1990–2000	269 (49)	175 (51)	25 (32)	15 (79)	54 (53)	
2001–2011	275 (51)	170 (49)	53 (68)	4 (21)	48 (47)	
Transplant No.						.55
First	488 (89)	311 (90)	69 (88)	19 (100)	89 (87)	
Second	53 (10)	31 (9)	9 (12)	0 (0)	13 (13)	
Third	3 (1)	3 (1)	0 (0)	0 (0)	0 (0)	
Cell source						<.001
Bone marrow	251 (46)	168 (49)	23 (30)	16 (84)	44 (43)	
Peripheral blood stem cell	269 (49)	167 (48)	46 (59)	3 (16)	53 (52)	
Cord blood	24 (4)	10 (3)	9 (11)	0 (0)	5 (5)	
Donor type						<.001
Autologous	104 (19)	71 (21)	16 (21)	0 (0)	17 (17)	
Related	202 (37)	135 (39)	23 (29)	8 (42)	36 (35)	
Unrelated	238 (44)	139 (40)	39 (50)	11 (58)	49 (48)	
Conditioning regimen[b]						.83
MAC	447 (82)	281 (82)	63 (78)	16 (84)	87 (85)	
RIC	97 (18)	64 (18)	15 (22)	3 (16)	15 (15)	
Days between transplant and PIV infection						.036
≤30	119 (22)	66 (19)	22 (28)	3 (16)	28 (28)	
31–365	349 (64)	238 (69)	40 (51)	14 (74)	57 (56)	
>365	76 (14)	41 (12)	16 (21)	2 (10)	17 (17)	
PIV type[c]						
PIV-1	52 (10)	26 (8)	16 (21)	1 (5)	9 (9)	
PIV-2	30 (5)	24 (7)	3 (4)	0 (0)	3 (3)	
PIV-3	434 (80)	275 (80)	53 (68)	17 (90)	89 (87)	
PIV-4	22 (4)	16 (5)	5 (6)	1 (5)	0 (0)	
Unclassified	6 (1)	4 (1)	1 (1)	0 (0)	1 (1)	
Quantitative viral load, median (range)	5.0×10^6 (1.0×10^2–1.1×10^9)			4.8×10^5 (1.0×10^2–3.3×10^8)	7.4×10^6 (2.7×10^3–1.1×10^9)	.51
Copathogen[d]						<.001
No	457 (84)	313 (91)	61 (78)	16 (84)	67 (66)	
Yes	87 (16)	32 (9)	17 (22)	3 (16)	35 (34)	
Oxygen at diagnosis						<.001
No	472 (87)	342 (99)	63 (81)	13 (68)	54 (53)	
Yes	72 (13)	3 (1)	15 (19)	6 (32)	48 (47)	
White blood cell count						<.001
>1000 cells/μL	447 (85)	299 (90)	59 (76)	19 (100)	70 (69)	
≤1000 cells/μL	82 (15)	32 (10)	19 (24)	0 (0)	31 (31)	

Table 1 continued.

Characteristic	Total (N = 544)	URTI (n = 345)	LRTD			*P* Value
			Possible (n = 78)	Probable (n = 19)	Proven (n = 102)	
Lymphocyte count						.026
>300 cells/μL	332 (64)	224 (68)	44 (58)	11 (58)	53 (53)	
≤300 cells/μL	191 (36)	104 (32)	32 (42)	8 (42)	47 (47)	
Neutrophil count						<.001
$>1000 \times 10^6$ cells/L	407 (77)	270 (82)	56 (73)	18 (95)	63 (63)	
$\leq 1000 \times 10^6$ cells/L	120 (23)	61 (18)	21 (27)	1 (5)	37 (37)	
Monocyte count						<.001
$>100 \times 10^6$ cells/L	381 (73)	266 (81)	53 (70)	11 (58)	51 (51)	
$\leq 100 \times 10^6$ cells/L	141 (27)	61 (19)	23 (30)	8 (42)	49 (49)	
Steroid dose before diagnosis[c]						
No	242 (47)	160 (50)	40 (53)	5 (26)	37 (38)	
<1 mg/kg	133 (26)	78 (24)	25 (34)	4 (21)	26 (26)	
1–2 mg/kg	124 (24)	77 (24)	9 (12)	7 (37)	31 (32)	
>2 mg/kg	13 (3)	5 (2)	1 (1)	3 (16)	4 (4)	
Ribavirin use[e]						<.001
No	483 (89)	336 (97)	73 (94)	12 (63)	62 (61)	
Yes	61 (11)	9 (3)	5 (6)	7 (37)	40 (39)	
IVIG use						<.001
No	373 (69)	251 (73)	58 (74)	11 (58)	53 (52)	
Low-dose[f]	138 (26)	89 (26)	17 (22)	3 (16)	29 (29)	
High-dose	31 (6)	4 (1)	3 (4)	5 (26)	19 (19)	

All values are indicated as No. (%). Additional baseline parameters (disease risk at transplant, graft-vs-host disease prophylaxis, recipient cytomegalovirus serostatus, percentage of forced expiratory volume in 1 second/forced vital capacity before PIV infection, and percentage of predicted total lung capacity before PIV infection) were examined and did not show statistical differences between groups.

Abbreviations: IVIG, intravenous immunoglobulin; LRTD, lower respiratory tract disease; MAC, myeloablative conditioning; PIV, parainfluenza virus; RIC, reduced-intensity conditioning; URTI, upper respiratory tract infection.

[a] Five patients with multiple transplants had their reference transplant after 2011.

[b] The MAC and RIC regimens were defined as previously described [14].

[c] Exact *P* value could not be calculated.

[d] A copathogen was defined as a significant pathogen detected in concurrent nasopharyngeal, bronchoalveolar lavage, or lung biopsy samples, or in a blood sample obtained within 2 days of diagnosis of PIV infection.

[e] Ribavirin was administered as follows: aerosolized in 45 patients, systemic in 10, both in 1, and unknown in 5.

[f] To maintain levels of >400 mg/dL, as needed.

Univariable analysis of risk factors for overall mortality demonstrated that probable and proven LRTD was significantly associated with higher mortality compared with URTI (hazard ratio [HR], 5.87; 95% confidence interval [CI], 2.7–12.8; $P < .001$ in probable cases, and HR, 9.23; 95% CI, 5.9–14.3; $P < .001$ in proven cases), whereas possible LRTD was not (HR, 1.49; 95% CI, .7–3.0; $P = .27$). Similar results were obtained in the univariable analysis of risk factors for mortality from respiratory failure (HR, 2.56; 95% CI, .9–7.6; $P = .09$ in possible cases; HR, 4.95; 95% CI, 1.1–22.9; $P = .041$ in probable cases; HR, 24.4; 95% CI, 11.9–50.0; $P < .001$ in proven cases). Because only 10 patients with possible LRTD and 8 with probable LRTD died by day 90 after diagnosis, it was not feasible to evaluate the impact of each disease category on mortality in multivariable analyses.

Hypoxemia and Hospitalization in Each LRTD Category

The probabilities of requiring mechanical ventilation by 28 days after PIV LRTD were 10%, 18%, and 41% in possible, probable, and proven cases, respectively ($P < .001$; Figure 2). Oxygen-free days in each group were shown in Table 3. Days alive without hospitalization by 28 days after PIV LRTD were longer in order of possible, probable, and proven cases (mean: 19 [SD, 10] days in possible cases, 15 [SD, 11] days in probable cases, 8.0 [SD, 9] days in proven cases, respectively, $P < .001$).

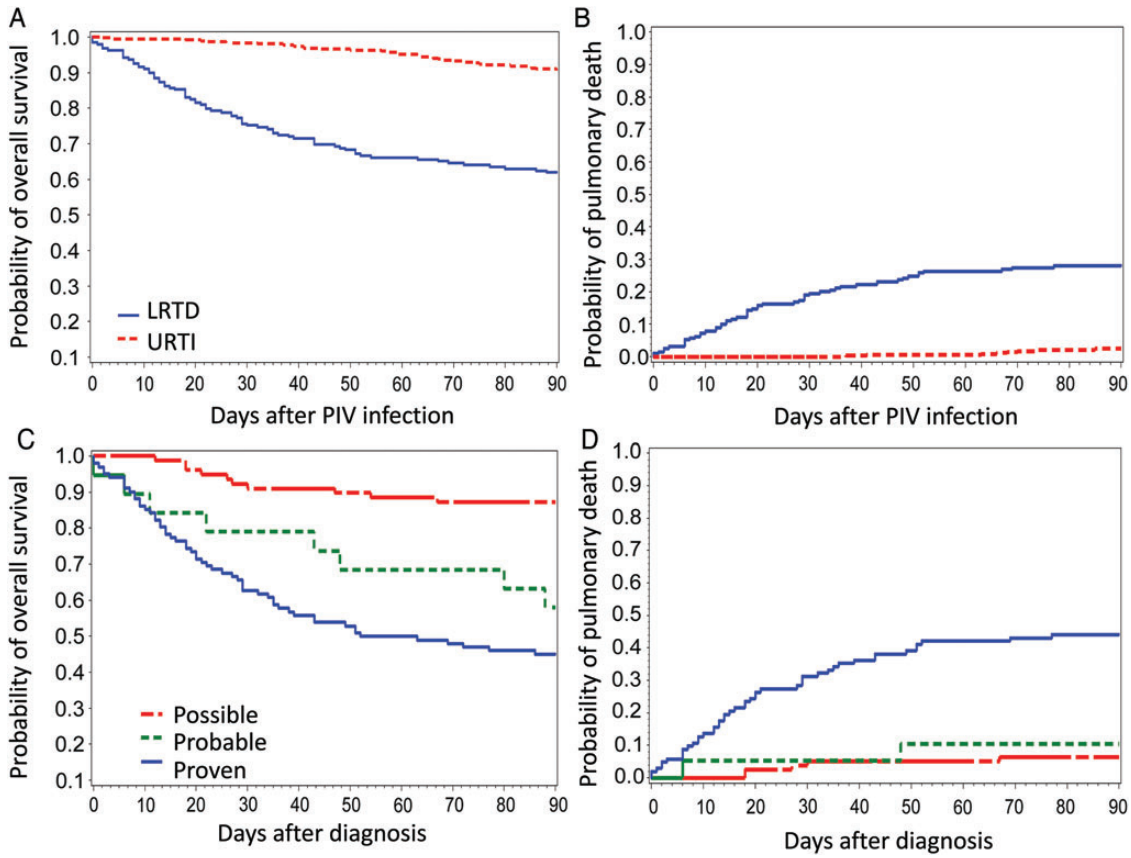

Figure 1. Probability of overall survival and death caused by respiratory failure. *A*, Kaplan-Meier estimate of overall survival according to classification of parainfluenza virus (PIV) infection in hematopoietic cell transplant (HCT) recipients ($P < .001$). *B*, Cumulative incidence of death caused by respiratory failure according to classification of PIV infection in HCT recipients ($P < .001$). *C*, Kaplan-Meier estimate of overall survival according to lower respiratory tract disease (LRTD) classification ($P < .001$). *D*, Cumulative incidence of death caused by respiratory failure according to LRTD classification ($P < .001$). Abbreviations: LRTD, lower respiratory tract disease; PIV, parainfluenza virus; URTI, upper respiratory tract infection.

Table 2. Mortality Rates in Each Group

		LRTD				
		Possible		Probable	Proven	
Category	URTI (n = 345)	(n = 22)	(n = 56)	(n = 19)	(n = 14)	(n = 88)
Positive nasopharyngeal test	Yes	Yes	Yes	[a]	[a]	[a]
Positive BAL/biopsy	NT/No	NT/No	NT/No	Yes	Yes	Yes
Positive radiography	No	Yes	Yes	No	Yes	Yes
Lower respiratory tract symptoms	[b]	No	Yes	Yes	No	Yes
Overall survival by day 90 (%)	91	100	82	58	57	43
Respiratory death by day 90 (%)	3	0	9	11	29	47

Abbreviations: BAL, bronchoalveolar lavage; LRTD, lower respiratory tract disease; NT, not tested; URTI, upper respiratory tract infection.

[a] Any result (yes or no) or nonnasopharyngeal test.

[b] Any result (yes or no).

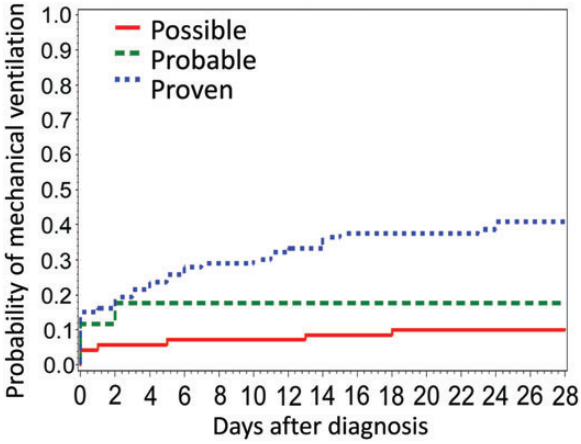

Figure 2. Probability of mechanical ventilation after parainfluenza virus lower respiratory tract disease (LRTD). Cumulative incidence of requirements of mechanical ventilation according to LRTD classification ($P < .001$).

Risk Factors for Mortality From All Causes or Respiratory Failure in Proven/Probable LRTD Cases

We focused on proven and probable LRTD cases to analyze the risk factors for mortality. A univariable analysis of risk factors for overall mortality showed that days between transplant and PIV infection, oxygen use, cell counts, and steroid dose >2 mg/kg/day before or after diagnosis were significant risk factors (Table 4). The same risk factors as well as PIV type were also significant for mortality from respiratory failure (Table 4). Among white blood cell populations, a monocyte count <100 cells/μL was identified as the most important factor for mortality in the multivariable analysis (Table 5). These results were similar to those obtained from patients with only proven LRTD, except that low monocyte counts become a significant risk factor for overall mortality in a multivariable analysis (adjusted HR [aHR], 2.01; 95% CI, 1.1–3.8; $P = .029$). Lower monocyte counts had a statistically significant effect on overall survival and risk of pulmonary death when analyzed in combination with oxygen requirements at diagnosis (Figure 3*A* and 3*B*).

A statistically significant difference in mortality was seen between probable and proven LRTD with symptoms (Table 4). The presence of clinical LRTD symptoms tended to be associated with worse outcome, although the effect did not reach statistical significance (Table 4).

Table 3. Oxygen-Free Days According to Parainfluenza Virus Lower Respiratory Tract Disease Category

Outcome	Possible	Probable	Proven	*P* Value
Any oxygen-free days				
By day 14 after PIV LRTD	12 (4)	10 (6)	6 (6)	<.0001
By day 28 after PIV LRTD	24 (8)	20 (11)	12 (11)	<.0001
>2 L/min oxygen-free days				
By day 14 after PIV LRTD	13 (3)	11 (4)	9 (4)	<.0001
By day 28 after PIV LRTD	26 (4)	22 (9)	18 (9)	<.0001

All values are presented as mean (standard deviation).

Abbreviations: LRTD, lower respiratory tract disease; PIV, parainfluenza virus.

Association Between Steroid Dose or Antiviral Treatment and Outcomes in Proven/Probable LRTD Cases

In the univariable analysis, steroid doses >2 mg/kg/day both before and after diagnosis of PIV LRTD were significantly associated with increased mortality, whereas steroid doses <2 mg/kg/day did not have any dose-dependent effect on mortality (Table 4). Because only a small number of the patients receiving steroid doses >2 mg/kg/day died by day 90 after diagnosis, the effect of steroid dose was evaluated while adjusting only for oxygen requirement at diagnosis, which is the most important risk factor for mortality. In this adjusted model, steroid dose >2 mg/kg/day after diagnosis remained significantly associated with mortality (Table 5).

The use of ribavirin was significantly associated with reduced overall mortality, but not with mortality from respiratory failure in multivariable analyses (Table 5). Among the patients with proven LRTD, however, ribavirin did not affect the overall or respiratory failure–related mortality (aHR, 0.64; 95% CI, .4–1.2; $P = .13$ in overall mortality, and aHR, 0.96; 95% CI, .5–1.8; $P = .91$ in respiratory mortality). High-dose intravenous immunoglobulin (IVIG) also had no effect on mortality after PIV LRTD (Table 5).

Association Between Diagnostic Method or BAL Viral Load and Outcomes in Proven/Probable LRTD Cases

Among 121 proven/probable LRTD cases, 31 (26%) patients were diagnosed using PCR alone. Although patients diagnosed with PCR alone had lower viral load ($P < .001$; Supplementary Figure 2*A*), there was no statistically significant survival advantage (Table 4).

In proven/probable LRTD cases, 48 stored BAL samples were available and tested by quantitative RT-PCR. There was no statistically significant difference between median viral load in subjects with proven and probable LRTD (Table 1, Supplementary Figure 2*B*). An association between viral load and mortality after PIV LRTD was not observed even after adjusting for oxygen use at diagnosis (HR, 1.03; 95% CI, .93–1.13; $P = .60$ for overall mortality, and HR, 1.09; 95% CI, .96–1.23; $P = .19$ for mortality from respiratory failure; Table 4 and data not shown).

DISCUSSION

This study demonstrates that the clinical outcome of patients with PIV detection in the lung (which we termed "proven" or

Table 4. Univariable Analysis of Risk Factors for Mortality From All Causes or Respiratory Failure by Day 90 After Parainfluenza Infection Among Proven/Probable Cases (n = 121)

	Overall Mortality			Mortality From Respiratory Failure		
Variables	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value
Disease category						
Probable	1.00			1.00		
Proven without symptoms	1.07	(.4–3.1)	.91	2.82	(.5–15.4)	.23
Proven with symptoms	1.61	(.8–3.4)	.21	5.19	(1.3–21.5)	.023
Presence of LRTD symptoms						
No	1.00			1.00		
Yes	1.39	(.6–3.2)	.44	1.54	(.6–4.3)	.41
Transplant year						
1990–2000	1.00			1.00		
2001–2011	0.62	(.4–1.0)	.07	0.70	(.4–1.3)	.23
Conditioning regimen						
MAC	1.00			1.00		
RIC	0.50	(.2–1.2)	.11	0.58	(.2–1.5)	.26
Days between transplant and PIV infection						
≤365	1.00			1.00		
>365	2.95	(1.2–7.4)	.020	5.46	(1.3–22.5)	.019
PIV type						
PIV-1, -2, -4	1.00			1.00		
PIV-3	0.63	(.3–1.2)	.18	0.44	(.2–.9)	.021
Quantitative viral load (log_{10})[a]	1.01	(.9–1.1)	.84	1.07	(.9–1.2)	.32
Diagnostic methods						
Conventional methods	1.00			1.00		
PCR alone	0.10	(.3–1.1)	.10	0.59	(.3–1.3)	.18
Copathogen						
No	1.00			1.00		
Yes	1.56	(1.0–2.6)	.08	1.76	(1.0–3.2)	.06
Oxygen at diagnosis						
No	1.00			1.00		
Yes	2.49	(1.4–4.5)	.002	4.25	(1.9–9.5)	<.001
Oxygen after diagnosis[b]						
≤2 L	1.00			1.00		
>2 L	2.09	(1.8–2.4)	<.001	2.62	(.9–7.4)	.07
Mechanical ventilation	2.59	(2.2–3.1)	<.001	17.93	(8.7–37.0)	<.001
White blood cell count						
>1000 cells/μL	1.00			1.00		
≤1000 cells/μL	2.27	(1.4–3.8)	.002	2.80	(1.6–5.1)	<.001
Lymphocyte count						
>300 cells/μL	1.00			1.00		
≤300 cells/μL	1.73	(1.1–2.9)	.032	1.82	(1.0–3.3)	.045
Neutrophil count						
>1000 cells/μL	1.00			1.00		
≤1000 cells/μL	2.35	(1.4–3.9)	<.001	2.71	(1.5–4.9)	<.001
Monocyte count						
>100 cells/μL	1.00			1.00		
≤100 cells/μL	2.30	(1.4–3.8)	.001	2.96	(1.6–5.4)	<.001

Table 4 continued.

	Overall Mortality			Mortality From Respiratory Failure		
Variables	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value
Steroid dose before diagnosis						
No	1.00			1.00		
<1 mg/kg	1.05	(.6–2.0)	.88	1.06	(.5–2.1)	.88
1–2 mg/kg	0.70	(.4–1.3)	.28	.62	(.3–1.3)	.22
>2 mg/kg	3.23	(1.5–7.2)	.004	1.41	(.4–4.8)	.58
Steroid dose after diagnosis[b]						
No	1.00			1.00		
<1 mg/kg	0.64	(.3–1.4)	.24	0.65	(.3–1.5)	.31
1–2 mg/kg	1.09	(.6–2.1)	.80	1.04	(.5–2.3)	.92
>2 mg/kg	4.39	(1.9–9.9)	<.001	4.12	(1.8–9.7)	.001
Ribavirin use[b]						
No	1.00			1.00		
Yes	0.68	(.4–1.1)	.15	0.95	(.5–1.7)	.88
IVIG use						
No/low-dose	1.00			1.00		
High-dose	1.17	(.7–2.1)	.60	1.29	(.7–2.5)	.44

All variables in Table 1 were used for the univariable analysis. Only variables with $P < .1$ in any analysis are shown in this table. The following parameters were also shown regardless of *P* values: presence of symptoms, quantitative viral load (log_{10}), ribavirin use, and IVIG use.

Abbreviations: CI, confidence interval; HR, hazard ratio; IVIG, intravenous immunoglobulin; LRTD, lower respiratory tract disease; MAC, myeloablative conditioning; PCR, polymerase chain reaction; PIV, parainfluenza virus; RIC, reduced-intensity conditioning.

[a] Viral titer was analyzed as a continuous variable.

[b] These variables are analyzed as time dependent.

"probable" LRTD) is significantly worse than that of patients with PIV detection only in upper respiratory tract samples (termed "possible" LRTD) (Figure 1*C* and 1*D*). Outcomes in patients with possible LRTD were similar to that of URTI. Our study also defined important clinical risk factors associated with poor outcomes of PIV LRTD, including oxygen requirement at diagnosis, low monocyte count, and steroid use >2 mg/kg/day.

Numerous studies of respiratory viruses in immunocompromised hosts have examined LRTD either as an endpoint or a risk factor for mortality. However, previously published results for both the incidence and risk factors for progression and outcome vary widely [3–6, 11, 12]. Data presented here suggest that the variance in definitions of LRTD is one important reason for the observed differences. To address this issue, we conducted the present study to define the optimized diagnostic criteria that correlate with outcome. Based on the site of virologic detection and clinical manifestations, we classified LRTD into 3 groups (possible, probable, and proven). The recently published Fourth European Conference on Infections in Leukaemia (ECIL-4) guidelines also proposed a classification based on levels of certainty (possible, probable, confirmed) [15]. However, the ECIL-4 classification is for all respiratory viruses, applies to both UTRI and LRTD, and uses symptoms, exposure, and any viral detection (independent of site) as classifiers. In contrast, our classification is strictly among LRTD cases that were all virologically confirmed [15]. In our paper, we specifically focused on the "possible" category, which includes patients with nasopharyngeal tests demonstrating PIV infection and abnormal chest radiography, but without confirmation of virus in the lower respiratory tract. Numerous publications as well as society guidelines have generally accepted that these patients have LRTD [5–8, 11, 15–19]. Our data demonstrate that the mortality in patients with possible LRTD is significantly better than that in patients with probable and proven disease (Figure 1*C* and 1*D*) and similar to that of patients with URTI (Table 2). The effect was persistent throughout the study period (data not shown). Interestingly, possible LRTD patients without symptoms tended to have even better outcome than URTI (Supplementary Figure 1). Nevertheless, many studies include this category as LRTD [6–8, 16, 17]. Therefore, we suggest that this group we defined as "possible" LRTD should be separated from the patient group with viral detection in the lung (proven and probable LRTD cases). We developed our definitions a priori based on clinical practices across centers and the spectrum of definitions used in the literature. The "possible" and "proven" categories are by far the most common categories documented, both in our series and elsewhere. Bronchoscopy based on PIV

Table 5. Multivariable Analysis of Risk Factors for Mortality From All Causes or Respiratory Failure by Day 90 After Diagnosis in Proven/Probable Cases (n = 121)

	Final Model			Steroid Dose			Ribavirin			IVIG		
	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value
Overall mortality												
Oxygen at diagnosis (yes vs no)	2.44	(1.3–4.4)	.003	2.19	(1.2–4.1)	.013	2.28	(1.3–4.2)	.007	2.63	(1.5–4.8)	.001
Days between transplant and PIV infection (≤365 vs >365)	2.26	(.9–5.8)	.09				2.46	(1.0–6.3)	.06	2.34	(.9–6.1)	.08
Transplant year (2001–2011 vs 1990–2000)	0.72	(.4–1.2)	.22				0.68	(.4–1.2)	.16	0.77	(.4–1.3)	.34
Monocyte count (<100 vs ≥100 cells/μL)	1.63	(.9–2.9)	.09				2.33	(1.4–4.0)	.002	1.97	(1.2–3.3)	.010
Neutrophil count (<1000 vs ≥ 1000 cells/μL)	1.58	(.9–2.8)	.12									
Steroid dose after diagnosis (>2 vs ≤2 mg/kg)[a]				3.80	(2.0–7.4)	<.001						
Ribavirin use (yes vs no)[a]							0.51	(.3–.9)	.021			
IVIG use (high-dose vs no/low-dose)										0.99	(.5–1.8)	.97
Mortality from respiratory failure												
Oxygen at diagnosis (yes vs no)	3.96	(1.7–9.1)	.001	3.59	(1.6–8.2)	.002	4.06	(1.8–9.3)	<.001	4.26	(1.9–9.7)	<.001
Days between transplant and PIV infection (≤365 vs >365)	4.14	(1.0–17.4)	.052				4.25	(1.0–17.9)	.049	4.13	(1.0–17.5)	.054
PIV type (PIV-3 vs PIV-1, -2, -4)	0.54	(.3–1.1)	.10				0.53	(.3–1.1)	.09	0.54	(.3–1.2)	.11
Monocyte counts (<100 vs ≥100 cells/μL)	2.07	(1.0–4.2)	.041				2.48	(1.3–4.7)	.006	2.34	(1.3–4.4)	.008
Neutrophil counts (<1000 vs ≥1000 cells/μL)	1.36	(.7–2.7)	.38									
Steroid dose after diagnosis (>2 vs ≤2 mg/kg)[a]				3.27	(1.5–6.9)	.008						
Ribavirin use (yes vs no)[a]							0.87	(.5–1.6)	.66			
IVIG use (high-dose vs no/low-dose)										1.09	(.5–2.2)	.81

Abbreviations: CI, confidence interval; HR, hazard ratio; IVIG, intravenous immunoglobulin; PIV, parainfluenza virus.

[a] These variables are analyzed as time dependent.

detection in the upper respiratory tract and the presence of LRTD symptoms or significant signs of obstruction by spirometry ("probable" category) was performed during the 1990s at our center, but it has become very uncommon in recent years. Nevertheless, these patients are informative as their outcome appears to be similar to that seen in proven cases.

Although our study focused on patients infected with PIV, we hypothesize that the outcome differences seen among the "possible" disease category may also exist for other respiratory viruses. Indeed, most studies and guidelines of non-PIV respiratory viruses include "possible" cases in their definitions of LRTD [7, 15–19]. Additional studies are needed to extend these results to other respiratory virus infections. More than a decade ago, the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) established the standard definitions for invasive fungal disease [20, 21]. These guidelines are widely accepted [22, 23], and resulted in improved reproducibility of study results between centers. These guidelines have greatly aided consensus endpoint definitions for clinical trials in invasive fungal disease. As new drugs for some respiratory viruses including PIV (eg, DAS181) are advancing through clinical development [24, 25], careful consideration of LRTD definitions that correlate with outcome is as important as clinical trial endpoints, entry criteria, and stratification variables.

Another novel observation of this study is that the monocyte count at diagnosis of LRTD appears to be critical to survival after PIV LRTD. Previous studies of respiratory viral infections in immunocompromised hosts suggest that lymphocytes or neutrophils are important cell components for mortality [5, 26]. In our study, however, lymphocyte counts appeared to be less important than monocyte or neutrophil counts, and monocytes seemed to comprise an important cell component (Tables 4 and 5). Indeed, patients who were both monocytopenic and required oxygen at diagnosis of LRTD had a

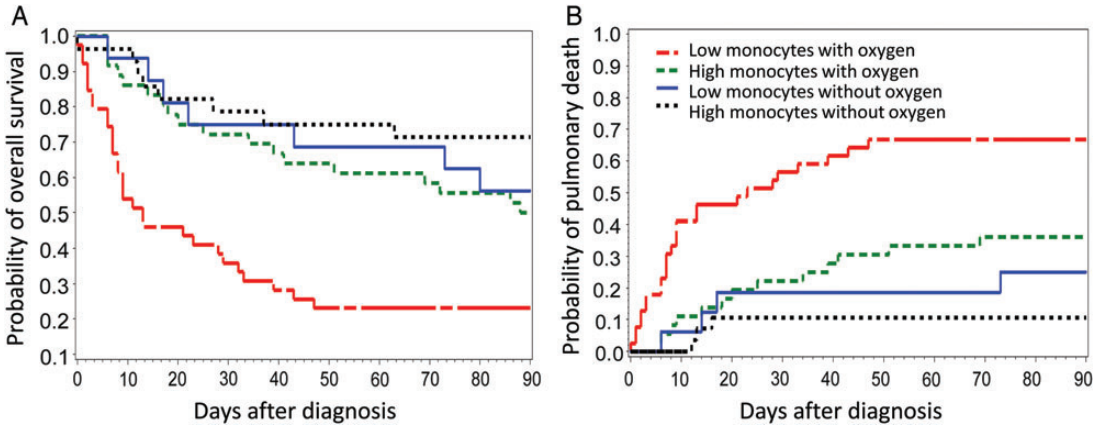

Figure 3. Probability of overall survival and death caused by respiratory failure according to monocyte count and oxygen requirement in proven/probable lower respiratory tract disease (LRTD) cases. *A*, Kaplan-Meier estimate of overall survival by monocyte count and oxygen requirement in proven/probable cases ($P < .001$). *B*, Cumulative incidence of death caused by respiratory failure according to LRTD classification in proven/probable cases ($P < .001$).

particularly high mortality (Figure 3). Monocytes have been known to be important to protect from viral infections [27–31]. Neutrophils may also have an important role at the time of pneumonia, and these 2 cells interact with each other [32–34]. These data from basic research support our finding that monocytes and neutrophils are important for outcomes after LRTD.

This is the first outcome study of PIV that used multivariable modeling for evaluating treatment strategies. Previous studies suggested that steroid use was associated with high mortality [5, 6, 12]. Our results showed that steroid use of ≤2 mg/kg/day after PIV LRTD was not associated with mortality. Based on our results, high-dose steroids for acute lung injury are not recommended, and a rapid taper of steroids for graft-vs-host disease may not be required when lower doses are used.

Because no antiviral drug is currently approved for the treatment of PIV disease, ribavirin and IVIG are often used off-label in PIV LRTD cases. Previous reports showed conflicting results ranging from no apparent efficacy on mortality after PIV LRTD [3–6, 12, 35], to moderate efficacy [36–39]. Most studies had a small sample size, and no statistical adjustments were made for disease severity. Thus, the effect of ribavirin is still controversial [40]. Our results show conflicting results for ribavirin treatment. Although the overall mortality model suggested a survival benefit among patients with proven and probable LRTD (Table 5), no significant effect was seen for death due to respiratory failure and for patients with proven LRTD. Some important variables such as high-dose steroids or the effect of combination therapy with IVIG could not be adequately assessed due to the small number of the patients with proven/probable LRTD who received these treatment combinations.

Recently, PCR testing has become more widely utilized as a diagnostic method and the frequency of diagnoses of viral infections has been increasing [13]. However, the association of quantitative RNA viral load on mortality after PIV LRTD has not been well evaluated. In our study, BAL samples that were also positive by conventional methods generally had a higher viral load (Supplementary Figure 2*A*), consistent with previous reports in nasal wash samples [13]. The results also show that the "proven" and "probable" disease categories do not differ with regard to viral load and that the presence of clinical LRTD symptoms was not associated with higher viral load (Supplementary Figure 2*B*). These findings provide support for combining both groups in outcome analyses, as proposed in this study. Our results did not identify RNA viral load in the BAL as a significant factor for mortality, although the number of subjects with available viral load was relatively small.

This study has several limitations. Although this study is the largest cohort of confirmed PIV infections in HCT recipients (which includes our previously reported cases [4] as well as subsequent cases through 2011), the sample size was still not sufficient to perform multivariable analyses to evaluate the impact of LRTD disease categories or the effect of combination of several drugs on mortality. Because of the retrospective nature of the study, BAL samples were available in only half of the patients for viral load quantification, which limited the multivariable modeling. The use of bronchoscopy for the workup of LRTD is largely based on protocols at our center, with almost all patients undergoing this procedure when radiographic signs or LRTD symptoms occur. Nevertheless, the final decision to perform bronchoscopy rests with the attending physician and some patients may not have undergone this procedure. Therefore, some probable or proven cases may have been missed, but this is unlikely to affect the major results of this study.

In conclusion, our data demonstrate that patients with PIV detection in the lungs (proven/probable LRTD) had worse outcomes compared with those with PIV detection in nasopharyngeal samples alone (possible LRTD). The outcome of possible LRTD was comparable to that of URTI. This LRTD definition could be useful for future outcome studies independent of virus types, and studies are needed to validate the results for other viruses and immunocompromised host settings. Consensus definitions in accordance with outcomes should be developed for respiratory viral disease.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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