

## ORIGINAL ARTICLE

# A parainfluenza-3 outbreak in a SCT unit: sepsis with multi-organ failure and multiple co-pathogens are associated with increased mortality

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**The estimated frequency of parainfluenza virus 3 (PIV-3) infections following haematopoietic SCT (HSCT) is 2–7%, whereas reported mortality ranges from 18 to 33%. We report a retrospective outcome analysis following an outbreak of PIV-3 infection in our transplant unit. A total of 16 HSCT patients developed PIV-3 infection. All patients had upper respiratory tract infection, whereas lower respiratory tract infection occurred in 8 patients. Overall, 13 patients were treated with aerosolised Ribavirin (2 g t.d.s. for 5 days) and i.v. Ig (0.5 g/kg) as per standard protocol. One patient refused treatment, whereas two patients with full immune reconstitution were not treated. Overall mortality was 62.5%. Sepsis with multi-organ failure and the presence of pulmonary co-pathogens were both significantly associated with PIV-3-related mortality. Our series confirms that high mortality is associated with PIV-3 infection in HSCT recipients. In patients who develop PIV-3 infection, despite strict enforcement of infection control policies, the best strategy might be careful risk assessment, with effective broad-spectrum anti-microbials in those who are at risk of secondary infection.**

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## Introduction

Respiratory virus infections are among the major causes of morbidity and mortality following haematopoietic SCT (HSCT). The reported frequency of symptomatic viral respiratory tract infection (RTI) varies between 3 and 8%,

with a significantly lower frequency seen following autologous compared with allo-HSCT.<sup>1,2</sup> This is likely to be an underestimate, as the incidence of respiratory virus infection diagnosed using new multiplex PCR techniques in one prospective study was 25%. However, this included both symptomatic and asymptomatic subjects.<sup>3</sup> The most frequently implicated viral pathogens are respiratory syncytial virus, influenza and parainfluenza viruses (PIVs).<sup>1,2</sup>

Parainfluenza viruses are well-recognised respiratory pathogens in children in whom infection is usually mild, ranging from the characteristic laryngotracheobronchitis (croup) to upper RTI (URTI), bronchiolitis and pneumonia. The lower respiratory tract typically is involved in only 15% of cases.<sup>4</sup> In the immunocompromised host, however, it is associated with significant morbidity and mortality. In one study, 86% of HSCT recipients with lower respiratory tract viral infection had severe airflow decline at 1 year after infection, although numbers were small.<sup>5</sup> This complication is associated with increased mortality risk and appears more frequent after PIV, lower RTI in particular.

The primary sites of infection are the mucous membranes of the nose and throat and the incubation period ranges from 2 to 4 days, with spread of virus throughout the respiratory epithelium demonstrable 1–3 days later.<sup>6</sup>

In England and Wales, PIV-3 accounts for the majority of PIV diagnoses and follows an annual epidemic cycle with a peak in late spring or summer.<sup>7</sup> Timing of infection in immunocompromised hosts mirrors that seen in the community.

The incidence of PIV infection post HSCT, diagnosed by the standard methods of direct immunofluorescence (DIF) and viral culture, varies from 2 to 9%.<sup>2,3,8–12</sup> However the incidence of PIV infection using PCR methods is higher, in one series up to 14%, although this also included asymptomatic cases.<sup>3</sup> The reported overall mortality associated with PIV-3 infection following HSCT ranges from 18 to 33%.<sup>8–10,12</sup> Only stem cell donation from an unrelated donor has been identified as a risk factor for PIV infection.<sup>8</sup>

We report an outbreak of PIV-3 infection in the HSCT unit at our centre over an 8-month period, and assess the overall mortality and contributing risk factors.

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## Patients and methods

### *Patient population*

Following an outbreak of PIV-3 infection in the Stem Cell Transplant Unit at Guy's and St Thomas' NHS Trust, a retrospective review of all HSCT patients diagnosed with PIV-3 between April and November 2008 was undertaken. Laboratory records were cross-referenced to ensure all cases of infection were included. Inclusion criteria were HSCT within 12 months of diagnosis (24 months in the presence of GVHD and/or continuing immunosuppressive therapy) and PIV-3 infection diagnosed by DIF assay on respiratory samples. Local policy was to actively screen for symptoms of infection. Respiratory samples were tested on any patient presenting to the ward or clinic in whom upper or lower respiratory tract symptoms were volunteered or detected.

### *Definitions*

URTI was defined as the acute onset of rhinorrhoea, sinusitis, pharyngitis or cough, without clinical or radiological evidence of lower respiratory tract involvement and/or hypoxia, together with detection of the virus in nasopharyngeal aspirate (NPA) samples.

Lower RTI (LRTI) was defined as clinical signs and symptoms of lower respiratory involvement with radiological evidence of new pulmonary infiltrates with or without hypoxia associated with the detection of the virus in NPA or bronchoalveolar lavage (BAL) specimens.<sup>1</sup>

A co-pathogen was defined as a bacterial, fungal or viral agent isolated from the respiratory tract within 7 days of the diagnosis of PIV-3 infection.

Community-acquired infection was defined as diagnosis of PIV-3 infection within 4 days of admission to hospital, given the 2–4 day incubation period of PIV-3. Nosocomial infection was defined as PIV-3 detected on a sample of 5 days or more after admission.

Lymphopenia was defined as an absolute lymphocyte count below  $1 \times 10^9$  cells/L.

Sepsis was defined clinically depending on the presence of fever, hypotension, tachycardia, oliguria and/or altered conscious state, but taking into account that the use of immune suppressants might mask typical signs of sepsis in this patient population. Positive blood cultures were not essential criteria for definition of sepsis.

Possible fungal infection was defined according to the presence of host factors and clinical features but the absence of mycological evidence (EORTC/MSG guidelines).<sup>13</sup>

### *Diagnostic methods*

Nasopharyngeal aspirate and/or BAL specimens were obtained from all HSCT patients with upper or lower respiratory tract symptoms. Nasopharyngeal aspirate was carried out by catheterisation of the nasopharynx, application of suction at 13–20 kPa, rolling of the catheter to increase cell collection and finally suction of a small amount of sterile saline to clear the sample into a container.

A standard one-step DIF technique was used for detection of PIV using a kit containing FITC-conjugated

monoclonal antibodies, with binding specific for PIV-1, 2 or 3 (Imagen Biotech, Manchester, UK). In all cases, samples were also examined for influenza, respiratory syncytial virus, metapneumovirus and adenovirus, and bacterial or fungal co-pathogens according to standard laboratory procedures.

### *Treatment*

All patients with PIV-3, detected by DIF, were considered for treatment with aerosolised Ribavirin at a dose of 2 g for three times daily for 5–7 days, and a single dose of i.v. Ig 0.5 g/kg administered on the day of admission. Treatment was started within 12 h of diagnosis in those with features of LRTI, lymphopenia (absolute lymphocyte count  $<1.0 \times 10^9$  cells/L), GVHD or those on steroids. Ribavirin was administered i.v. when nebulised Ribavirin could not be safely delivered and at the treating physicians' discretion. Clearance of infection was confirmed by two negative samples spaced at least 7 days apart.

Targeted antibiotics, anti-fungal and anti-viral drugs were used following identification of specific pathogens, whereas broad-spectrum antibiotics were used to treat systemic sepsis, pending pathogen identification.

### *Statistical methods*

Descriptive and comparative statistics were performed using IBM SPSS Statistics 18 (SPSS, Chicago, IL, USA). Fisher's exact test was used for testing significance of several categorical variables associated with risk of PIV-3 infection or PIV-3 LRTI and for comparing frequency of sepsis, coinfection, LRTI and mortality according to type of virus infection (PIV-3 versus respiratory syncytial virus).

## Results

### *Patient data*

A total of 16 patients were identified as having PIV-3 infection between April and November 2008. Their clinical characteristics at presentation, and details of transplant type and conditioning are documented in Table 1. No other subtypes of PIV were detected in this period.

Infection was community acquired in 6 cases and hospital acquired in 10 cases, with community-acquired infection accounting for four of the first five cases to be diagnosed. The median time from transplant to initial detection of PIV was 210 days (range 3–568).

In total, 15 patients had received allo-HSCT (2 myeloablative, 13 non-myeloablative conditioning (NMA)), although 1 patient undergoing autologous HSCT contracted nosocomial infection. Stem cell source was sibling donor in 3 patients and volunteer-unrelated donor in 12 of the allo-HSCT recipients. Conditioning included fludarabine in 12/16 cases and alemtuzumab in 13/16 cases. Infection occurred pre-engraftment in three patients.

Total lymphocyte count was measured in all patients on diagnosis of PIV-3 and was  $<1 \times 10^9$  cells/L in 15/16 patients. CD4 count was measured within 4 weeks of infection in 13/16 patients and was  $<200$  cells/ $\mu$ L in 12 patients.

Initial presentation was with coryzal symptoms suggestive of URTI in 13 patients and productive cough or

**Table 1** Demographics and patient data

Patient/treatment data	No. (%)
Median age in years (range)	56 (22–66)
Men (%)	9 (56)
<i>Donor type</i>	
Autologous	1 (6)
Matched sibling	3 (19)
Unrelated	12 (75)
<i>Underlying disease</i>	
Acute leukaemia	6 (38)
Chronic leukaemia	1 (6)
Non-Hodgkin's lymphoma	5 (31)
Myeloma/AL amyloid	3 (19)
Myelofibrosis	1 (6)
<i>Conditioning</i>	
Non-myeloablative	13 (81)
Myeloablative	2 (12)
Campath-1H	11 (69)
<i>Acute GVHD</i>	8 (50)
Grades 0–1	2 (12)
Grades 2–4	6 (38)
<i>Steroids, no (%)</i>	
< 1 mg/kg	1 (6)
> 1 mg/kg	6 (38)
<i>Immunosuppressive drugs (n)</i>	
0	5 (31)
1	6 (38)
2	3 (19)
3	2 (12)
<i>Time of diagnosis (days after transplant)</i>	
< 100	6 (37)
100–365	7 (44)
> 365	3 (19)
<i>Lymphopenia</i>	
< $1 \times 10^9$ cells/L	14 (88)
> $1 \times 10^9$ cells/L	2 (12)
<i>CD4 count</i>	
< 200	12 (75)
> 200	1 (6)
N/A	3 (19)
Ribavirin treatment	13 (81)
i.v. Ig	13 (81)
<i>Infection site at presentation</i>	
URTI	8 (50)
URTI then LRTI	5 (31)
LRTI	3 (19)

Abbreviations: LRTI=lower respiratory tract infection; URTI=upper respiratory tract infection.

hypoxia suggestive of LRTI in 3 patients. The median time from transplant to presentation was 210 days (range 3–568). Five patients developed LRTI after starting nebulised Ribavirin treatment for URTI. In all eight cases of LRTI, new pulmonary infiltrates were seen on the chest radiograph.

### Diagnosis

Infection was diagnosed by DIF in all 16 cases. A total of 11 cases were diagnosed following NPA alone, 2 cases on

BAL alone (one of whom was PIV-3 negative on NPA, 5 days before the bronchoscopy), and 3 cases on both NPA and BAL. Of these three cases, two had PIV-3 infection diagnosed by NPA, but bronchoscopy was performed because of worsening respiratory function to confirm PIV-3 LRTI and search for other pathogens. PIV-3 infection had not been diagnosed in the third case until the patient was admitted to the intensive care unit with rapid onset sepsis and respiratory failure at which point NPA and bronchoscopy was carried out.

Infection persisted for a median of 22 days (7–59) in the nine patients who cleared infection (as defined by two consecutive negative NPA or BAL samples spaced at least 7 days apart). Persistent infection occurred in 7 cases, all of whom died.

### Treatment

Out of 16 patients, 13 were treated with nebulised Ribavirin for 5 days. Ribavirin was administered i.v. to one patient following intubation on admission to the intensive care unit. It was decided not to treat two patients, who were more than 1 year post transplant, with either CD4 count > 200 or lymphocyte count >  $1 \times 10^9$  cells/L, no features of LRTI, no GVHD and no co-infection, both of whom went on to clear infection. One patient refused treatment and died with multi-organ failure.

Single-dose i.v. Ig (0.5 g/kg) was given to all patients who were treated with Ribavirin.

Five patients required ventilatory support, four needed invasive treatment and one patient needed non-invasive treatment, for progressive respiratory failure.

In total, 11 patients were treated with broad-spectrum antibiotics, the indication being either a systemic inflammatory response or admission to the intensive care unit for ventilatory support. Antibiotics were not started routinely on admission for Ribavirin treatment.

### Co-infection

Co-pathogens were grown from the sputum or from BAL samples in nine cases. The range of organisms detected and the temporal relationship with PIV diagnosis are shown in Table 2. Fungal infection was proven in three and possible in two, based on host factors and new infiltrates on computed tomography imaging. Reactivation of CMV was detected on BAL in two cases. In all cases of 'sepsis', blood cultures 14 days before and after the diagnosis of PIV-3 were negative.

### Outcome

Overall mortality was 62.5% (10/16) of which seven deaths were directly attributable to PIV-3 infection and its complications (44%). A 30-day mortality was 12.5% (2/16), and both deaths were secondary to complications of PIV-3 infection. Eight deaths occurred within 100 days of PIV-3 infection and five were attributed to complications of PIV-3 infection. Three patients cleared infection and recovered from this acute episode but died of disease recurrence ( $n = 1$ ) or sepsis ( $n = 2$ ) soon after.

Infection persisted for a median of 22 days in patients who went on to clear infection, as defined by two negative NPAs spaced at least 7 days apart.

**Table 2** Co-pathogens

Patient no.	Diagnosis of PIV (days after transplant)	Abs lymphocyte count	Immune suppression	GVHD (grade)	Bacteria	Timing relative to PIV diagnosis (days) (before PIV-, after PIV+)	Source	Fungal	Timing relative to PIV diagnosis	Source	Viral	Timing relative to PIV diagnosis	Source
3	260	0.3	Prednisolone (>2 mg/kg) CYA MMF	IV	Coliforms	-8	Sputum				CMV	0	BAL
					<i>Pseudomonas aeruginosa</i>	-1	Sputum						
6	195	0.2	Prednisolone (>2 mg/kg) CYA MMF	IV	<i>Klebsiella</i>	-8	Sputum	<i>Aspergillus fumigatus</i>	-2	Sputum			
			Sirolimus ECP		<i>Pseudomonas aeruginosa</i>	-13	Sputum						
7	107	0.1	Prednisolone (>2 mg/kg)	IV	<i>Stenotrophomonas maltophilia</i>	-4	Sputum						
8	13	0	CYA None	—	Mixed organisms	8	BAL	<i>Candida</i> spp.	8	BAL			
9	43	0.2	CYA	—	Upper respiratory tract flora	-1	BAL	Possible	-6	CT scan			
11	225	0.4	Prednisolone (>2 mg/kg) CYA ECP	III	Mixed organisms including coliforms	-8	Sputum						
13	568	0.7	Prednisolone (<1 mg/kg)	—	<i>Haemophilus influenzae</i>	5	BAL	Possible	3	CT scan			
					<i>Staphylococcus aureus</i>	5	BAL						
14	328	1	Prednisolone (>2 mg/kg) MMF	III	<i>Stenotrophomonas</i>	-1	Sputum						
					<i>Enterobacter cloacae</i>	-1							
16	227	0.2	Prednisolone (>2 mg/kg) MMF Etanercept	IV	<i>Escherichia coli</i>	1	Sputum	<i>Aspergillus fumigatus</i>	30	Sputum			

Abbreviations: BAL = bronchoalveolar lavage; CT = computed tomography; MMF = Mycophenolate Mofetil; PIV = parainfluenza virus.

Sepsis with multi-organ failure (RR 48;  $P=0.001$ ) and the presence of pulmonary co-pathogens (RR 1.96,  $P=0.024$ ) were both significantly associated with PIV-3-related mortality. LRTI was associated with PIV-3-related mortality (RR 2.32,  $P=0.059$ ). Two out of three patients infected pre-engraftment died. Grades 3–4 GVHD, use of Campath, volunteer-unrelated donors or low CD4 counts were not significantly associated with death.

## Discussion

In our patient series, a high-mortality rate was seen despite a low threshold for early testing for respiratory viral infection in symptomatic individuals. The three factors associated with poor outcome were sepsis/multi-organ failure, pulmonary co-pathogens and LRTI. Analysis of factors predisposing to LRTI did not identify any significant results.

The limitations of our study are that analysis is retrospective and the sample size is small. We did not undertake sampling of asymptomatic patients who had been in contact with confirmed cases as this was not policy in our

unit, NPA being considered too invasive. Quantification of PIV-3 viral load was not undertaken.

Nucleic acid amplification technology (NAT) for detection of respiratory viruses is increasingly used to detect a wider range of respiratory viruses, such as parainfluenza virus-4, bocavirus, rhinovirus, coronaviruses and enteroviruses, and offers increased sensitivity for detection of PIV-3, with positive PCR demonstrated in DIF-negative subjects.<sup>14</sup>

Although the overall mortality associated with PIV-3 infection in several large prospective and retrospective studies ranged from 18 to 33% (see refs 8, 10–12), a significant increase to 46–75% was observed following LRTI.<sup>2,8,12</sup> Although not demonstrated here, the use of high-dose systemic steroids and lymphopenia have been identified as important risk factors for progression of URTI to LRTI in PIV infection.<sup>8,11</sup>

PIV-3, as with other respiratory viruses, may predispose to secondary bacterial infection by epithelial damage, impairment of ciliary function and triggering of host-inflammatory responses. In addition, bacterial infection may be promoted by increased expression of bacterial receptors on PIV-3-infected cells.<sup>15</sup> The presence of pulmonary co-pathogens and the need for mechanical

ventilation increase mortality in PIV LRTI.<sup>8</sup> A single English retrospective study found the frequency of LRTI in PIV-3-infected patients to be 58% (see ref. 12) in comparison with 23 and 24% in two retrospective series obtained from the USA.<sup>2,8</sup> Two reports of small PIV-3 outbreaks in English HSCT populations found the frequency of LRTI to range from 25 to 52%, although in contrast to the outcome in our cohort, this was not associated with an increase in overall mortality.<sup>11,16</sup>

At the time of this outbreak, our standard screen for detection of respiratory viruses was by DIF on NPA samples. Owing to the poor tolerability of this test procedure, patients were screened for clinical features of respiratory infection and samples collected only from those with symptoms. Although it has been postulated that active surveillance and early treatment could account for low mortality,<sup>17</sup> we saw a high-mortality rate, despite an active screening policy in symptomatic patients. In the case of our series, it is likely that the underlying immune deficiency, assessed by total lymphocyte count, presence or absence of GVHD and use of high-dose steroids allowed the development of secondary infections, which were often fatal despite appropriate anti-microbial therapy.

Although the overall incidence of respiratory virus infection is similar following myeloablative and NMA conditioning, the progression from URTI to LRTI has been found to be lower following NMA conditioning.<sup>2</sup> However, a 35% cumulative probability of respiratory virus infection has been reported following use of NMA conditioning, including Campath-1H, with PIV-3, was the most frequently isolated organism (45.7% of cases) but a low-mortality rate of 8%.<sup>12</sup> In our series, the majority of patients were conditioned with NMA protocols, and Campath-1H was part of the conditioning regimen in 81% of patients. Thus, although we could not comment on the effect of conditioning type, the use of Campath-1H may have contributed to the high frequency of PIV-3 LRTI.

Effective infection control procedures including isolation of at-risk patients in the outpatient clinic should be enforced, especially as the four subjects with community-acquired infections were likely to have come into contact in the outpatient department. Studies have identified close-contact transmission and surface contamination as more important than droplet spread or direct hand contact in PIV transmission, and infectious virus has been demonstrated for up to 10 h on skin and cloth.<sup>18,19</sup>

At present, no drugs are licensed by the Food and Drug Administration for the treatment of PIV infection in children or adults. In the absence of robust clinical efficacy data from large randomised controlled studies and with no licensed treatment available, some centres offer Ribavirin on the basis of evidence of *in vitro* and *in vivo* activity against parainfluenza viruses in case reports and small series.<sup>8,12,20</sup> Sialic acid analogues, such as 4-Guanidino-Neu5Ac2en (Zanamivir), designed to interfere with the binding of the viral HA-NA to host sialic acid residues, were originally designed to inhibit influenza infections but are only partially active in blocking PIV-3 HN *in vitro*<sup>21</sup> and there is no evidence of *in vivo* activity. Analogous compounds that block the haemagglutinin and neuraminidase of PIVs 1–3, such as BCX 2798 and BCX 2855,

show great promise, although trials in humans are yet to begin.<sup>22,23</sup>

When the EBMT sent out a survey to assess the frequency and type of infection outbreaks in the 505 registered Stem Cell Transplant Centres between 1991 and 2001, replies were received from 41 centres and only 13 of these reported infection outbreaks.<sup>24</sup> Out of 23 outbreaks of infection reported, 8 were viral, of which 5 were PIV-3, all in reports obtained from the UK transplant centres. Following the introduction of the joint accreditation committee-ISCT (Europe) and EBMT (JACIE)-run independent quality management scheme, reliable monitoring and reporting of post-HSCT complications across Europe should become mandatory.

Since this outbreak we more actively question patients for symptoms and signs of viral upper or LRTI following HSCT. We do not currently undertake screening of asymptomatic individuals, but in the event of another outbreak we would be in a position to screen staff and patients. Following the introduction of nucleic acid amplification technology, with easier and less-invasive sample acquisition, utilising nose and throat swabs rather than NPA, infection with a broader range of pathogens might be identified at an earlier stage. In- and outpatient staff in the transplant unit are trained to identify patients with respiratory symptoms and testing of swabs allows rapid and sensitive diagnosis of infection.

Potential bacterial and fungal pathogens are actively excluded and any features of a systemic inflammatory response prompt the early use of broad-spectrum antibiotics. Parenteral broad-spectrum antibiotics are also actively considered for all suspected PIV-3 cases with GVHD, on high-dose steroids or with lymphopenia. Respiratory isolation of all patients with suspected and proven infection continues to be routine practice.

However, the area of PIV3 infection post HSCT is difficult to monitor. Many patients are at home in the community where there might be asymptomatic shedding, screening is expensive and finally there continues to be no effective treatment.

### Conflict of interest

The authors declare no conflict of interest.

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