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Short communication

Human rhinovirus C in adult haematopoietic stem cell transplant recipients with respiratory illness

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ABSTRACT

Background: A previously unidentified species of human rhinovirus, HRV-C, was described in 2006 in association with lower respiratory tract infection (LRTI). Features of infection in immunosuppressed adults are poorly characterised.
Objectives: This study aims to determine the epidemiology of HRV-C in haematopoietic stem cell transplant (HSCT) recipients in a single centre.
Study design: A prospective cohort study of all HSCT recipients admitted to Westmead Hospital, Westmead, Australia from 1 July 2005 to 30 September 2007 was undertaken. Nose/throat samples were collected from all patients at the time of admission and patients developing pre-defined symptoms and/or signs of respiratory infection during the admission. Samples were processed and tested for rhinoviruses and 14 other respiratory viruses using nucleic acid-based methods, immunofluorescence and culture. HRV genotyping was performed by sequencing a region of the rhinovirus 5′ untranslated region (UTR). Clinical data on each episode were collected prospectively.
Results: HRVs were identified in 24 episodes: 8% of 299 episodes of clinically- defined respiratory infections and 39% of 61 episodes in which respiratory viruses were detected. HRV-C was most frequent (HRV-C: nine, HRV-A: eight and HRV-B: two). Seven episodes of HRV-C, five with pneumonia, occurred within 100 days of HSCT. Co-pathogens were frequent.
Conclusions: The newly described HRV-C was the most common rhinovirus group detected in HSCT recipients with respiratory infection, with co-pathogens being frequent. Further research is required to understand the activity and pathogenicity of this virus in HSCT recipients.

1. Background

A previously unidentified human rhinovirus (HRV) species, HRV-C, was described in 2006[1,2] in association with lower respiratory tract infection and wheezing in children.[3,4] Features of infection in adults are poorly described. While HRVs are the most frequent respiratory viruses detected by polymerase chain reaction (PCR) in adult haematopoietic stem cell transplant (HSCT) recipients,[5,6] the epidemiology of HRV-C in this population is unknown.

2. Objectives

This study aims to describe the clinical epidemiology of HRV-C in a cohort of adult HSCT recipients with acute respiratory infection.

3. Study design

A prospective cohort study of HSCT recipients admitted to Westmead Hospital, Westmead, Australia, between 1 July 2005 and 30 September 2007 was undertaken. This centre performs 40–55

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allogeneic and 15–25 autologous adult HSCTs annually. Institutional ethics approval was granted.

The nursing staff completed a daily check list comprising six clinical features of possible respiratory virus infection: cough; fever >38 °C in the last 24 h; sneezing or runny nose; dyspnoea; oxygen saturation <95% on air; and clinician-documented crackles on chest examination.[7] Paired nose/throat swabs (NTSs) were collected into a viral transport medium (Copan Diagnostics, Murrieta, CA, USA) when ≥2 clinical features were present, and from all patients on admission to the haematology ward regardless of symptoms. Bronchoalveolar lavage (BAL) was performed at the discretion of treating physicians. Repeated samples were collected only if symptoms persisted.

Immunofluorescence was performed for influenzaviruses A and B (IFVs), parainfluenzaviruses (PIV) 1–3, respiratory syncytial virus (RSV), human adenovirus (HAdV; Chemicon Inc., Temecula, CA, USA) and human metapneumovirus (HMPV; D3DFA, Diagnostic Hybrids, Athens, OH, USA) detection (Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW, Australia). NTS negative by IFV, and all BAL samples, were cultured for viruses.

A sample aliquot was stored at −80 °C for PCR for HRVs,[2,8] IFVs, PIV 1–3, RSV, HMPV, HAdV, coronaviruses OC43, 229E, NL63 and HKU1, polyomaviruses WU and KI and human bocavirus[3,4,7] (Queensland Paediatric Infectious Diseases Laboratory, Herston, QLD, Australia). Samples were batched, with results not available to clinicians. A region of the HRV 5′ untranslated region (UTR) was sequenced, with a type assigned when it shared ≥97% nucleotide identity with the same region of a fully sequenced HRV using methods described previously (Table 1).[9] Briefly, 2 μl of nucleic acid extract was reverse transcribed in a total 20 μl reaction volume (OneStep RT-PCR kit, QIAGEN, Australia; 50 °C for 60 min), subjected to a hot-start (95 °C for 15 min) then a ∼380 bp complementary DNA (cDNA) was amplified using 45 cycles of 94 °C for 20 s, 55 °C for 20 s and 72 °C for 50 s with a final incubation at 72 °C for 10 min. The product from positive reactions was subjected to nucleotide sequencing using the PRISM BigDye sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA). Sequence analysis on products ranging from >200 nucleotides (nt) in length was

Table 1
Oligonucleotide sequences of conventional HRV screening.

Oligonucleotide Name	Oligonucleotide sequence (5′–3′)
OL26 01.1	GCACTTCTGTTTCCCCC
OL26 02.1	CGGACACCCAAAGTAG

Table 2
Clinical details of all rhinovirus episodes ($n = 27$).

Patient (episode)	HRV	Gender (age, years)	HSCT type (stem cell source)	Time post-HSCT	Clinical syndrome	Co-pathogen	GVHD	Immunosuppression
1 (1)	A	M (58)	Pre-autologous (PBSCT)	−4 days	URTI	Nil	N/A	Nil
2 (1)	A	M (42)	Pre-MUD (PBSCT)	−14 days	URTI	Nil	N/A	MTX, MP
3 (1)	A	F (33)	MR RIC (PBSCT)	7 months	U&LRTI	Yes[a]	Yes	Prednisolone, tacrolimus, sirolimus
4 (1)	A	M (62)	Autologous (PBSCT)	23 months	Asymptomatic	Nil	No	Nil
5 (1)	A	M (28)	MUD (Cord)	15 months	LRTI	Yes[a]	Yes	CSA
6 (1)	A	M (36)	MUD (Cord)	29 months	URTI	Yes[a]	Yes	Tacrolimus
7 (1)	A	M (65)	MR (BMT)	152 months	LRTI	Nil	No	Cyclophosphamide, vincristine, doxorubicin, glivec
8 (1)	A	M (48)	MUD (PBSCT)	22 days	URTI	Nil	Yes	CSA, MTX, MP
9 (1)	B	M (52)	MR RIC (BMT)	13 days	U&LRTI	Nil	No	CSA
10 (1)	B	F (21)	MR (PBSCT)	12 months	LRTI	Yes[c]	No	Prednisolone, tacrolimus, sirolimus
11 (1)	C	M (19)	MUD (Cord)	59 days	LRTI	Nil	No	CSA
12 (1)	C	M (56)	MUD (PBSCT)	4 days	LRTI	Yes[d]	No	CSA, MTX, MP
13 (1)	C	M (24)	MUD (Cord)	12 days	U&LRTI	Yes[e]	Yes	CSA, MP
14 (1)	C	M (18)	MUD RIC (PBSCT)	4 days	U&LRTI	Yes[g]	No	CSA, MP, MMF, alemtuzumab
15 (1)	C	M (51)	Pre-MR RIC (PBSCT)	−23 days	Asymptomatic	Nil	N/A	Nil
16 (1)	C	M (20)	MUD (BMT)	47 months	URTI	Nil	No	Tacrolimus, prednisolone
16 (2)	C	M (20)	MUD (BMT)	49 months	LRTI (fatal)	Yes[f]	No	CSA, prednisolone
17 (1)	C	M (63)	MR (PBSCT)	76 days	URTI	Nil	No	CSA, MP, dacluzimab, infliximab, MMF
18 (1)	C	F (54)	MUD RIC (PBSCT)	39 days	URTI	Nil	No	CSA
19 (1)	Untypeable	M (61)	MUD RIC (PBSCT)	7 months	URTI	Nil	Yes	CSA
20 (1)	Untypeable	M (49)	MR (PBSCT)	7 days	URTI	Nil	Yes	CSA, MTX, MP
20 (2)	Enterovirus	M (49)	MR (PBSCT)	78 days	URTI	Nil	No	CSA, prednisolone
21 (1)	Untypeable	M (53)	Autologous (PBSCT)	−7 days	Asymptomatic	Nil	No	Nil
22 (1)	Untypeable	M (48)	MR (PBSCT)	28 months	LRTI	Nil	No	Nil
23 (1)	Positive not repeated	M (53)	MR (PBSCT)	7 days	LRTI	Nil	Yes	CSA, MTX
24 (1)	Double infection	M (35)	MR (PBSCT)	29 days	URTI	Nil	Yes	CSA
25 (1)	N/A[b]	M (32)	MUD (PBSCT)	7 months	URTI	Nil	No	Nil

BMT: bone marrow transplant; CSA: cyclosporine A; MMF: mycophenolatemofetil; MP: methylprednisolone; MR: matched related donor; MTX: methotrexate; MUD: matched unrelated donor; N/A: not available; PBSCT: peripheral blood stem cell transplant; RIC: reduced intensity conditioning; a second episode of HRV infection requires a seven day period free of symptoms and hypoxia, resolution of imaging changes in those with LRTI; and a respiratory sample negative for the previously identified virus.

[a] Adenovirus.
[b] PCR negative, culture positive.
[c] Probable invasive aspergillosis and *H. influenzae* (BAL).
[d] *E. faecium* & *C. glabrata* in blood cultures.
[e] Polyomavirus KI & *A. xyloxidans* bacteraemia.
[f] Polyomavirus KI & *E. coli* bacteraemia.
[g] Polyomavirus KI.

Table 3
HRV species with associated clinical features.

		A (no. = 8)	B (no. = 2)	C (no. = 9)	Total (no. = 19)
Demographics	Male:female	7:1	1:1	7:2	15:4
	Median age years (range)	63 (33–65)	37 (21–52)	24 (18–63)	39 (18–65)
Graft type	Allogeneic recipient	5	2	8	15
Donor	Related donor	2	2	1	5
	Unrelated donor	3	0	7	10
Acquisition	Pre or during HSCT admission	3	1	3	7
	During conditioning or <100 days	3	1	7	11
	Nosocomial acquisition	2	1	5	8
Clinical illness	Asymptomatic	1	0	1	2
	URTI	4	0	3	7
	LRTI	2	1	3	6
	U&LRTI	1	1	2	4
	All LRTI	3	2	5	10
	No LRTI	5	0	4	9
	Wheeze	0	1	2	3
	Co-pathogen	3	1	4	8
	Co-viruses	3	0	3	6
Outcomes	Respiratory support	0	2	2	4
	Fatal outcome	0	0	1	1

Nosocomial acquisition – onset following four or more days of hospitalization.

conducted using Geneious Pro.[10] This assay has been shown to be at least as clinically sensitive as others widely used.[11] If an HRV was repeatedly positive in the screening assay but no useful sequence could be obtained it was called untypeable. Sequences containing two picornavirus templates (a member of an enterovirus (EV) and HRV species) that could not be further interpreted were designated "double" infections. A *post hoc* analysis of all HRV samples and review of clinical data were undertaken.

4. Definitions

4.1. Upper respiratory tract infection (URTI)

The presence of two or more of rhinorrhoea, sneezing or cough, with a normal chest examination and radiological imaging (chest X-ray or computed tomography).

4.2. Lower respiratory tract infection (LRTI or pneumonia)

The presence of fever and hypoxia or pulmonary infiltrates reported on radiological imaging.

4.3. Nosocomial acquisition

Symptom onset 4 or more days following hospital admission, in keeping with previous definitions.[12,13]

4.4. Co-pathogens

Other respiratory viruses (including those detected by PCR), bacteraemia or candidaemia detected from samples collected during the episode of respiratory infection.

5. Results

Respiratory virus testing was performed in 213 of the 299 episodes of respiratory tract information (RTI) from 193 HSCT recipients. In symptomatic patients, the first sample was collected a median of 2 days (range 0–5) following symptom onset. At least one respiratory virus was detected in 61 episodes, with HRV in 24 (39%; 12 URTIs, 12 LRTIs, including four with upper respiratory symptoms). Asymptomatic infection was detected from four of 205 (2.0%) samples (three episodes).

Phylogenetic analysis was performed on the 26 HRVs identified by PCR; the viral species was identified in 19 (73%). One culture-positive, PCR-negative isolate was unavailable for sequencing. Four

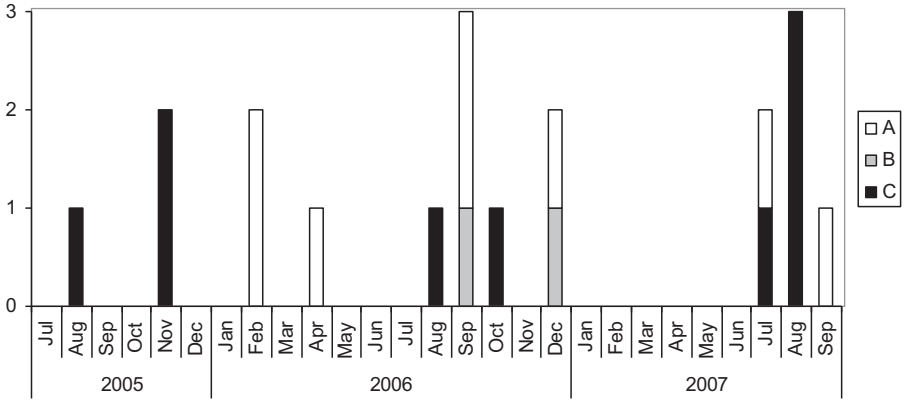

Fig. 1. Epidemic curve of HRV-A, -B and -C.

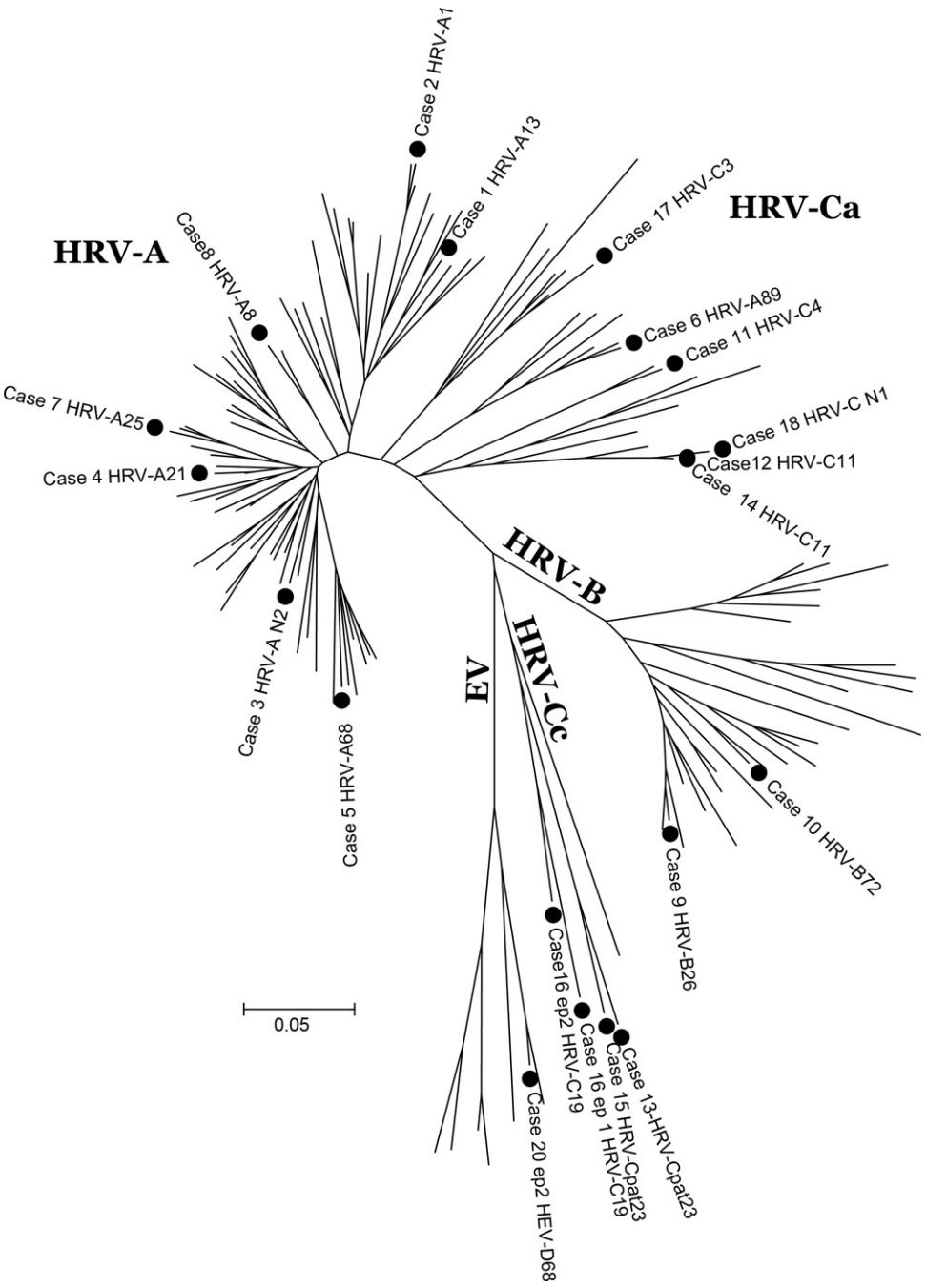

Fig. 2. Topology tree of detected HRV-C.

HRVs were untypeable, one showed double infection, one was not reproducible and another was identified as EV-D68 (Table 2).

HRV-C was most frequent (nine, 47%), followed by HRV-A (eight, 42%) and HRV-B (two, 11%) (Tables 2 and 3). Pneumonia was present in five of nine episodes with HRV-C (56%), both with HRV-B, and three of eight with HRV-A. Infection was acquired nosocomially in eight episodes. Co-pathogens were present in seven episodes, with co-viruses in six (Table 2). Invasive ventilation was required in two HRV-C episodes (patients #10 and #12), both with co-pathogens. Patient #10 died from overwhelming sepsis with *Haemophilus influenzae* bacteraemia and probable invasive aspergillosis within 24 h of symptom onset.

While episodes occurred over 26 months (Fig. 1), a cluster of four cases of HRV-C occurred in July–August 2007. The phylogenetic tree (Fig. 2) shows the same strain (C11) causing two of these episodes; these were nosocomially acquired and related in time and place (cases #12 and #14).

6. Discussion

The newly described HRV-C was present in almost half of all episodes of rhinovirus infection in this cohort. This follows a report of HRV-C predominance in a cohort of Thai children hospitalised with LRTI.[14] HRV-A has dominated cohorts of adults and children hospitalised with RTI, with HRV-C in 25–35% of episodes.[4,15–18] This includes renal transplant recipients and others with immunocompromise.[15,16]

Although HRV-C and HRV-A caused a similar number of episodes, HRV-C had a higher proportion with pneumonia (56% and 38%, respectively), occurring in the early post-transplant period

(78% and 38%) and associated with co-pathogens (44% and 25%). An association with HRV-C, but not HRV-A, and pneumonia in adults with underlying medical conditions or advanced age has been noted,[18] but not in all cohorts, including outpatient renal transplant recipients.[16] Seven of the nine episodes of HRV-C occurred within 100 days of HSCT when patients have severe immunosuppression and high infection risk, particularly post allogeneic transplant.

While HRV-B and HRV-C were more commonly associated with pneumonia, in only one episode was HRV-C the sole pathogen. The presence of co-pathogens in earlier HRV-C studies is limited to co-virus detections in 8% in adults[17] and 26% in children,[19] with a similar proportion in this cohort. Polyomavirus KI was the most frequent co-virus; however, its clinical importance remains uncertain.[20]

This study is limited by the small number of HRV detected from symptomatic samples compared with 30% detection in a prior HSCT cohort[6] in which routine weekly testing occurred within the first 100 days post HSCT, and BAL sampling was frequent in pneumonia. In our study, 52 BAL samples were collected from 223 episodes of pneumonia. A diagnostic BAL was obtained in only one of 12 HRV-associated pneumonias, with the remaining episodes inferred from positive upper respiratory samples. It is recognised that upper respiratory samples are less sensitive in pneumonia[21] and HRV in this setting may be missed. We acknowledge that without lower respiratory samples for histopathology and further microbiologic testing we cannot state definitively that HRV-C caused pneumonia.

In summary, HRV-C was detected in adult HSCT recipients with viral RTIs including pneumonia, with the poorest outcomes in the presence of co-pathogens. Patients appear to be most at risk during the early post-HSCT period when immunosuppression is greatest. Further characterisation of the epidemiology and pathogenicity of HRV-C in other geographically representative adult HSCT populations is warranted.

Competing interests

All authors report no conflicts of interest relevant to this article.

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Ethical approval

Ethical approval was given by Western Sydney Area Health Service Ethics Committee, HREC2006/2/4.32(2309).

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