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Initial High Viral Load Is Associated with Prolonged Shedding of Human Rhinovirus in Allogeneic Hematopoietic Cell Transplant Recipients

Chikara Ogimi[1,2,3], Hu Xie[4], Wendy M. Leisenring[4], Jane M. Kuypers[1,5], Keith R Jerome[1,5], Angela P. Campbell[1,2,3,†], Janet A. Englund[2,3], Michael Boeckh[1,4,6], Alpana Waghmare[1,2,3,*]

[1] *Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington*
[2] *Department of Pediatrics, University of Washington, Seattle, Washington*
[3] *Pediatric Infectious Diseases Division, Seattle Children's Hospital, Seattle, Washington*
[4] *Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington*
[5] *Department of Laboratory Medicine, University of Washington, Seattle, Washington*
[6] *Department of Medicine, University of Washington, Seattle, Washington*

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A B S T R A C T

Recent data suggest human rhinovirus (HRV) is associated with lower respiratory tract infection and mortality in hematopoietic cell transplant (HCT) recipients. Examining risk factors for prolonged viral shedding may provide critical insight for the development of novel therapeutics and help inform infection prevention practices. Our objective was to identify risk factors for prolonged shedding of HRV post-HCT. We prospectively collected weekly nasal samples from allogeneic HCT recipients from day 0 to day 100 post-transplant, and performed real-time reverse transcriptase PCR (December 2005 to February 2010). Subjects with symptomatic HRV infection and a negative test within 2 weeks of the last positive were included. Duration of shedding was defined as time between the first positive and first negative samples. Cycle threshold (Ct) values were used as a proxy for viral load. HRV species were identified by sequencing the 5′ noncoding region. Logistic regression analyses were performed to evaluate factors associated with prolonged shedding (≥21 days). We identified 38 HCT recipients with HRV infection fulfilling study criteria (32 adults, 6 children). Median duration of shedding was 9.5 days (range, 2 to 89 days); 18 patients had prolonged shedding. Among 26 samples sequenced, 69% were species A, and species B and C accounted for 15% each; the median shedding duration of HRV did not differ among species ($P = .17$). Bivariable logistic regression analyses suggest that initial high viral load (low Ct value) is associated with prolonged shedding. HCT recipients with initial high viral loads are at risk for prolonged HRV viral shedding.

INTRODUCTION

The impact of respiratory viral infections in hematopoietic cell transplant (HCT) recipients is widely appreciated [1–3]. Human rhinoviruses (HRVs) have been increasingly recognized as serious pathogens that are frequently associated with lower respiratory tract infection, mortality and prolonged shedding [4,5]. There is a need for effective antiviral therapy in this population, and new therapeutics are under investigation [6,7]. Defining risk factors for prolonged shedding may provide important information to risk-stratify subjects in antiviral clinical trials with viral load endpoints. Furthermore, risk factors for prolonged viral shedding may inform effective infection prevention practices. The objective of this study was to evaluate viral and host factors associated with prolonged HRV shedding in the upper respiratory tract in HCT recipients.

METHODS

Subjects

We conducted a prospective surveillance study of patients undergoing allogeneic HCT at the Fred Hutchinson Cancer Research Center from December 2005 to February 2010 [8]. For the first 100 days post-transplant, weekly standardized respiratory symptom surveys and viral PCR testing on upper respiratory samples was performed. Only subjects with respiratory symptoms at the time of first HRV detection in the 100 days post-HCT were included in the current study. Subjects were included only if the end of the viral shedding period could be defined, which was if a negative viral PCR test result occurred within 2 weeks of the last positive test [9]. The study was approved by the Institutional Review Board at Fred Hutchinson Cancer Research Center.

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* Correspondence and reprint requests: Alpana Waghmare, MD, Pediatric Infectious Diseases Division, Seattle Children's Hospital, 4800 Sand Point Way NE, MA.7.226, Seattle, WA 98105.
E-mail address: alpana.waghmare@seattlechildrens.org (A. Waghmare).
† Present affiliations: Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; and Epidemiology and Prevention Branch, Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

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Laboratory Testing and Definitions

HRV RNA was detected in upper respiratory tract specimens by nucleic acid extraction and real-time reverse transcriptase PCR (RT-PCR). Cycle threshold (Ct) was used as an inverse proxy for viral load, with lower Ct correlating with higher viral load. Sanger sequencing was performed on the 5′ noncoding region using saved nasal samples [10]. The shedding duration was defined as time between the first positive and first negative samples, and prolonged shedding was defined as shedding ≥21 days [9]. Highest daily steroid dose and lowest cell counts in the 2 weeks before first HRV detection were recorded.

Statistical Analysis

Covariates were selected from previously identified risk factors for prolonged shedding of human coronavirus and disease progression of other respiratory viruses, as well as important biological variables thought to influence shedding duration [9,11,12]. Continuous covariates (age and Ct) were analyzed as continuous and dichotomous (above and below the median) variables. Univariable and bivariable logistic regression analyses were performed to evaluate associations between covariates and prolonged shedding. Variables with a P value ≤.2 in univariable models were candidates for bivariable models. Mann-Whitney U and Kruskal-Wallis tests were performed to compare continuous values between 2 and more than 2 groups, respectively. Two-sided P values <.05 were considered statistically significant. All statistical analyses were performed using SAS 9.4 for Windows (SAS Institute, Cary, NC).

RESULTS

Host and Virologic Characteristics

We identified 38 HCT recipients with HRV infection fulfilling study criteria (32 adults and 6 children) (Table 1). Approximately one half of patients were leukopenic in the 2 weeks before virus detection, but only 1 patient received high-dose steroids (≥1 mg/kg/day). The median duration of shedding was 9.5 days (range, 2 to 89 days) and 18 patients (47%) had prolonged shedding (≥21 days). Among 18 patients with Ct values below the median (30.3), 13 (72%) had prolonged shedding. Among 18 patients with prolonged shedding, 2 progressed to lower respiratory tract infection based on positive HRV PCR from bronchoalveolar lavage specimens (on days 21 and 31) during the shedding period; no patient with shorter shedding progressed. Among 26 sequenced nasal samples, 69% were species A, and 15% each were species B and C. The median shedding duration did not differ among species (P = .17) (Supplementary Figure S1). Another 45 patients were asymptomatic at the time of first detection of HRV and the median initial viral load of these 45 patients did not differ from that of the 38 symptomatic patients (P = .92). Among the 45 initially asymptomatic patients, 18 subsequently developed respiratory symptoms during the shedding period (time to development of symptoms: median 14 days; interquartile range, 7 to 37 days) but no patient progressed to lower respiratory tract infection.

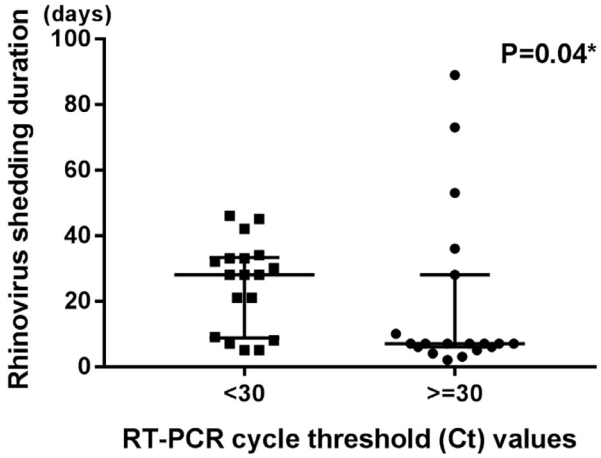

Figure 1. Shedding duration of human rhinovirus by Ct values (n = 38).

Outcome Analyses

Higher viral load (lower Ct value) at onset was associated with longer shedding duration (Figure 1 and Table 1). Bivariable logistic regression analyses indicated that initial Ct below the median (30.3) was consistently associated with prolonged shedding in all models (Figure 2). Similar analyses evaluating Ct as a continuous variable showed consistent results with the exception that the number of transplants was not statistically significant (data not shown). Nonmyeloablative conditioning was associated with prolonged shedding; however, it was no longer statistically significant after adjusting for age at transplantation or number of transplants

Table 1
Characteristics of Allogeneic HCT Recipients with Rhinovirus Upper Respiratory Tract Infection

	Total (N = 38)	Patients with Prolonged Shedding (n = 18)	Patients with Short-Term Shedding (n = 20)
Female	13 (34)	5 (28)	8 (40)
Age, yr	50 (1-71)	56 (1-71)	39 (1-62)
Second transplant	7 (18)	5 (28)	2 (10)
Donor type			
Related	19 (50)	10 (56)	9 (45)
Conditioning regimen			
Nonmyeloablative	18 (47)	12 (67)	6 (30)
Viral onset following transplantation			
<30 d	13 (34)	2 (11)	11 (55)
Rhinovirus species			
A	18 (47)	10 (56)	8 (40)
B	4 (11)	2 (11)	2 (10)
C	4 (11)	2 (11)	2 (10)
Unknown	12 (32)	4 (22)	8 (40)
Ct value	30.3 (22.4-39.4)	28.7 (22.4-38.7)	33.5 (24.2-39.4)
Lowest WBC <1,000 cells/μL*	18 (47)	7 (39)	11 (55)
Lowest lymphocyte count <100 cells/μL*	17 (45)	9 (50)	8 (40)
Lowest neutrophil count <500 cells/μL*	18 (47)	8 (44)	10 (50)
Lowest monocyte count <100 cells/μL*	23 (61)	11 (61)	12 (60)
Highest daily steroid dose*			
<1 mg/kg	37 (97)	18 (100)	19 (95)

Data are presented as n (%) or median (range).
* In the 2 weeks before first rhinovirus detection.

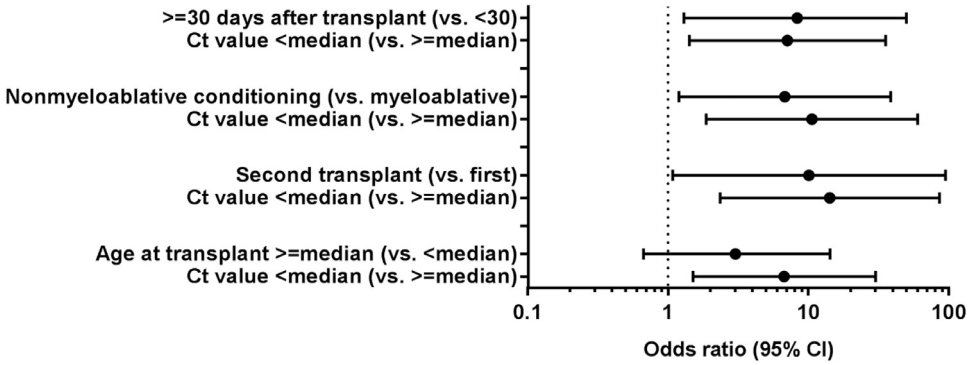

Figure 2. Odds ratios and 95% confidence intervals from bivariable models evaluating Ct value below median as a risk factor for prolonged shedding (n = 38).

(adjusted odds ratio [95% confidence interval], 3.7 (.9 to 15.3) and 4.1 [.9 to 19.4], respectively).

DISCUSSION

In a cohort of allogeneic HCT recipients with prospective upper respiratory tract sampling, initial high viral load was associated with prolonged shedding of HRV. The shedding duration appeared to be similar across all 3 HRV species.

With the widespread use of molecular diagnostics, HRV has been increasingly reported as a serious pathogen in immunocompromised hosts [13–16]. HRV has been demonstrated to be common in the lower respiratory tract, with mortality after lower respiratory tract infection comparable to that seen with well-established respiratory pathogens including respiratory syncytial virus, parainfluenza virus, and influenza [4]. Among respiratory viruses, HRV is prone to shed for long periods in transplant recipients [5]. Prolonged viral shedding can potentially contribute to HRV transmission and be of particular concern for infection prevention, although hospital outbreaks have been mainly reported for other respiratory viruses [17–22]. In the current study, 2 patients developed lower respiratory tract infection during periods of prolonged shedding. Our study did not have a large enough sample size to analyze the effect of prolonged shedding on adverse clinical outcomes; further studies are needed to clarify whether prolonged shedding significantly affects clinical outcomes.

The growing concern regarding poor outcomes associated with HRV infection following HCT has led to recognition of the need for antiviral agents [6,7]. Viral shedding duration is often used as an endpoint in clinical trials of new antivirals [23–25], and identifying risk factors for prolonged shedding is thus critical for appropriate stratification of patients in randomized trials. However, data on factors associated with prolonged shedding of respiratory viruses are limited, with the exception of influenza virus in mainly immunocompetent populations and coronavirus in HCT recipients [9,26,27]. The present study showed initial high viral load was a risk factor for prolonged HRV shedding in HCT recipients, consistent with our previous study of coronavirus [9].

This study, somewhat unexpectedly, showed that nonmyeloablative conditioning was associated with prolonged shedding after adjusting for viral load. We hypothesized that this association was partly due to nonmyeloablative conditioning being more common in older patients and those undergoing a second transplant. Both groups are more likely to have prolonged shedding, and conditioning regimen was not statistically significant after adjusting for these factors.

A limitation of our study is the relatively small sample size, which allowed us to perform only bivariable logistic regression analyses; however, the association between initial viral load and prolonged shedding appeared to be robust as it was shown consistently in different models. We were able to perform sequencing in only two-thirds of patients, primarily due to low viral load. Thus, our ability to detect small differences in shedding duration among HRV species was limited. Quantification of HRV using RT-PCR assays with a consensus HRV primer and probe set may be limited due to the diversity of HRV genotypes; reverse transcriptase digital PCR has shown more accurate quantification [28]. However, the overall performance of RT-PCR appears to be similar to reverse transcriptase digital PCR for most genotypes. Low Ct value, dichotomized at the median, was consistently associated with prolonged shedding in all models. Therefore, different quantification methods are unlikely to significantly impact our conclusion. Finally, we defined the end of shedding as the first negative virus detection regardless of whether patients continued to have respiratory symptoms. It is possible that shedding at viral loads below the limit of detection was present, but we felt that this definition would be more objective than using the duration of symptoms because ongoing symptoms cannot be necessarily attributed to persistent HRV infection.

In summary, initial high viral load is a risk factor for prolonged shedding of HRV in HCT recipients, an important finding for the future design of randomized clinical trials of novel therapeutics with viral load endpoints. These data may also inform effective infection control policies, as the expected HRV shedding duration is $\geq$21 days in patients with high viral loads.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.07.006.

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