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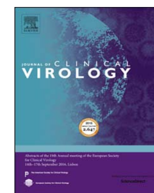

Full length article

Rhinovirus viremia in adult patients with high viral load in bronchoalveolar lavages

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ABSTRACT

Background: In children, rhinovirus viremia has been associated with higher nasopharyngeal loads and increase in severity of clinical signs and symptoms.
Objectives: This study aims to detect rhinovirus viremia in adult patients and to establish potential correlations with the clinical course.
Study design: Adult patients with rhinovirus strongly positive bronchoalveolar lavages (BAL, quantitation cycle, Cq values < 25) detected between 2008 and 2014 were studied retrospectively. Blood sampled between two weeks before and two weeks after BAL sampling was tested for rhinovirus RNA. Underlying conditions, symptoms, radiography, microbiological data, and disease outcome were analysed.
Results: Twenty-seven of 43 patients with rhinovirus positive BAL at Cq values < 25 had blood samples available within the prespecified time-frame (mean blood 3–4 samples per patient). Four of these 27 patients (15%) tested rhinovirus RNA positive in their blood (of whom one patient twice). Genotyping demonstrated rhinovirus A01, A24, B52 and B92 in these four immunocompromised patients.

Viremic patients were not significantly different with regard to underlying conditions, respiratory symptoms, radiological findings, co-pathogens nor the number of blood samples tested for RV. However, patients with rhinovirus viremia had significant higher mortality rates compared to patients without viremia, as all four died as a consequence of respiratory problems (100%) versus 22% (5/23), p = 0.007 (Fisher's exact).
Conclusions: Rhinovirus viremia can occur in adult patients with a high viral load in BAL fluid. Rhinovirus viremia may be considered a negative prognostic factor, although a causative role with regard to the adverse outcome has yet to be demonstrated.

1. Background

Rhinovirus (RV) is the most common virus found in respiratory tract infections in children and adults [1–3]. Rhinovirus primarily results in mild upper respiratory tract infections, known as the "common cold". However, a role for rhinovirus in lower respiratory tract infections has also been described. Rhinovirus was detected in 2–17% of the community acquired pneumonias [4–8], in children even up to 26% [9]. Severe rhinovirus-associated pneumonias may givesimilar symptoms and rates of mortality as severe influenza-associated pneumonia, but are more likely to occur in immunocompromised [10]. Generally, the clinical picture of rhinovirus infections appears to be more severe in patients with a higher viral load and in elderly patients, immunocompromised hosts and patients with underlying chronic lung diseases [11–15]. Although rhinovirus C has been implicated in more severe disease [16], other studies failed to show differences in clinical picture between different rhinovirus species [17–19].

In several case reports and prospective case series, rhinovirus RNA has been demonstrated in blood of paediatric patients [20–27]. Approximately 12% of the children with rhinovirus respiratory infections were found to be viremic on admission. This percentage dropped to 7% on day three after admission [23,25]. Of the viremic paediatric patients, the majority were infected with rhinovirus C, varying from 67 to 87% [20,23]. Viremia was associated with a history of asthma, higher nasopharyngeal viral loads and more severe clinical signs and symptoms [20,25]. In addition, case reports on rhinovirus viremia have been published in patients with fatal outcome, suggesting a correlation with more severe disease [21,26,27]. Rhinovirus viremia in adult patients

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has not yet been demonstrated. Rhinovirus RNA was analysed in adult patients, inoculated with rhinovirus-16, by deMore et al. [28] This group found no evidence of viremia. However this were healthy subjects, or with mild asthma, with upper respiratory tract symptoms not requiring hospitalisation.

2. Objectives

The aim of this study was to determine whether rhinovirus viremia occurs in adults and whether there is a relationship with the clinical course of the infection.

3. Study design

3.1. Patients and samples

A laboratory database (GLIMS, MIPS, Belgium) search was performed for bronchoalveolar lavages (BAL) in adults between January 2008 and June of 2014 in the tertiary care hospital Leiden University Medical Center (LUMC, Leiden, the Netherlands). All BAL samples were included in the initial selection, however this invasive diagnostic procedure is performed primarily in respiratory insufficient and immunocompromised patients. Adult patients with a rhinovirus PCR positive BAL and a quantitation cycle (Cq) value of 25 and below were considered strongly positive, described as risk factor for RV viremia in children [20], and included in the study. Of these patients, stored plasma (-80 °C) or serum (-20 °C) samples (previously sent to the microbiological lab for routine diagnostics) were collected (serum when plasma was not available). All samples available in the time period from two weeks before to two weeks after BAL sampling, with a maximum of one per day, were tested for the presence of rhinovirus RNA.

Patient characteristics, underlying diseases, symptoms, laboratory values, chest radiography, and disease outcome were retrospectively obtained from the patient records and laboratory databases. Stem cell transplantation patients were routinely screened for reactivation of cytomegalovirus (CMV), Epstein Barr virus and adenovirus by viral load testing.

3.2. Rhinovirus RNA detection

Nucleic acids were extracted from thawed serum or plasma samples with the MagNA Pure LC, using the Total Nucleic Acid Isolation Kit − Large Volume (Roche Diagnostics). The input was 1000 µl serum or plasma with elution of 50 µl extracted nucleic acid. If insufficient clinical sample was available, negative plasma was added up to 1000 µl.

Rhinovirus RNA amplification was performed in duplicate with an in-house real-time polymerase chain reaction (amplifying a 142-bp fragment of the 5′-UTR region), all samples in one run simultaneously, using primers, probes and conditions previously described by Loens et al. [29].Cq values, normalized using a fixed fluorescence threshold, were used as an indicative measure of viral loadRhinovirus typing of respiratory samples from viremic patients was performed by amplification and sequencing of the VP4/VP2 genome region as previously described by Zlateva et al. [30].

3.3. Statistical analysis

Underlying respiratory illnesses, transplantation status, symptoms, radiographic changes, microbiologic findings, and outcome of rhinovirus viremia positive patients were compared to rhinovirus viremia negative patients. Categorical data were compared using 2 × 2 tables with Fisher's exact test and Odds ratios. Numeric variables were compared using the independent *t*-test for equality of means.

All statistical analysis was performed using IBM SPSS Statistics version 20.00 software for Windows.

3.4. Ethical approval

The study design was approved by the medical ethics review committee of the LUMC.

4. Results

4.1. Patient selection

Between January 2008 and June of 2014, 791 bronchoalveolar lavages from 638 adult patients were tested for rhinovirus RNA. A total of 114 BAL samples from 84 (13%) patients tested positive for rhinovirus (range Cq values 15–45, randomly distributed, data not shown). Of 43 patients (51%, 48 BAL samples), the RV PCR result had a Cq < 25. A total of 84 blood samples were available from 27 of these 43 patients (63%), on average three- four blood samples per BAL sample (31 BAL samples). In 14 samples from nine patients, the available plasma volume varied from 200 to 950 µl.

4.2. Rhinovirus viremia positive patients

Four out of the 27 patients (15%) with BAL RV Cq < 25 tested rhinovirus RNA positive in their blood samples (range Cq values 34–42) divided over the years 2010, 2011 and 2012. From these viremic patients a total of 22 blood samples were tested of which five tested positive. One patient was found viremic twice, on day −six and day +three relative to BAL sampling. For an overview of tested blood samples relative to the day of the BAL see Fig. 1.

4.3. Genotyping of RV viremic patients

The rhinovirus load in the blood samples of the viremic patients was considered too low to enable successful genotyping. Therefore, the associated BAL samples were used, which resulted in two patients with RV-A: A24 and A01, and two patients with RV- B: B52 and B92.

4.4. Characteristics of rhinovirus viremic patients

Patient 1 (Fig. 1), was a 60-year-old female who received allogeneic stem cell transplantation for multiple myeloma. One month after transplantation she was admitted at the haematology department with neutropenic fever with a leukocyte count of 0.1×10^9 g/L. During her stay in the hospital she developed cough and dyspnoea. One day after symptom onset a BAL was performed, which was positive for rhinovirus A01 (Cq 24), parainfluenza 2 (Cq 15), adenovirus (Cq 35) and tested galactomannan positive. Bacterial culture showed *Stenotrophomonas maltophilia*. One of six blood samples tested RV positive, Cq 42. Fourteen days after the BAL was performed the patient died with multiple, respiratory and systemic infections (CMV reactivation- Aspergillus pneumonia- Stenotrophomonas bacteraemia) and unexplained on-going neutropenia. No autopsy was performed. The last two blood samples before her death were RV negative.

Patient 2, was a 68-year-old male with myelodysplastic syndrome for which he received non-myeloablative allogeneic stem cell transplantation four months prior to admittance to the intensive care unit (ICU) due to respiratory failure. At that time leukocyte count was 3.6×10^9/L and lymphocyte count 0.6×10^9/L. Four days after symptom onset a BAL was performed, in which a rhinovirus RV-B52 (Cq 23), was detected. No other pathogen (viral, bacterial, parasitic or fungal) could be found in the BAL sample. In total three blood samples were tested. The blood samples drawn six days before and three days after the BAL tested rhinovirus positive, Cq 34–36. The blood sample drawn three days before the BAL was rhinovirus negative. The patient died 11 days after the BAL was performed. Autopsy showed extensive pulmonary fibrosis, the lung architecture was completely destroyed without specific characteristics. Reported cause of death was

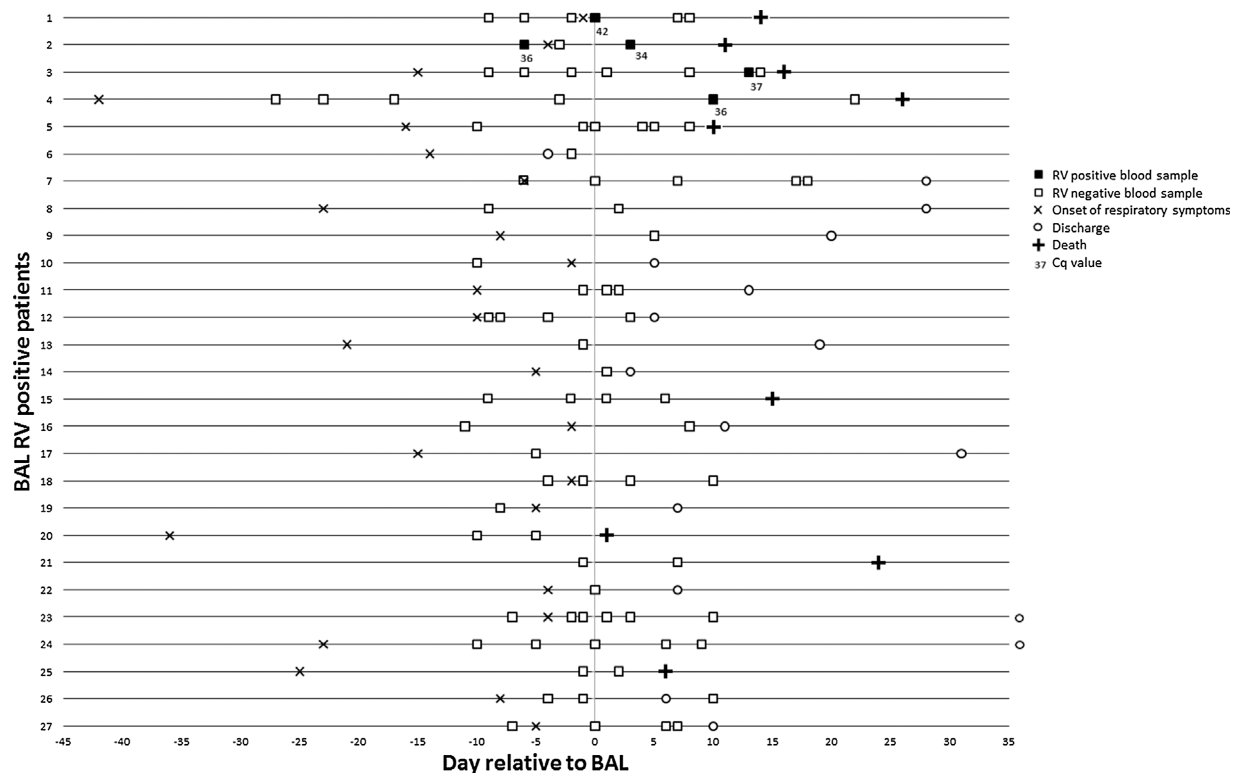

Fig. 1. Detection of rhinovirus in blood relative to the bronchoalveolar lavage (day 0).
This figure shows the distribution of the different samples compared to the sample date of the bronchoalveolar lavage, the onset of symptoms, and the day of discharge/death of the patients. Of patients 3, 4 and 7 multiple BAL samples were included. In this figure the first BAL was shown as day 0 if two BALs were available (patients 3 and 7), or the second BAL of three in total (patient 4).
Horizontal lines represent individual patients; the squares represent individual blood samples, onset of respiratory symptoms, discharge, and death. Cq values are shown for the positive samples.
Abbreviations: RV: rhinovirus, BAL: bronchoalveolar lavage.

respiratory failure of unknown cause.

Patient 3 was a 65-year-old female with a double cord blood stem cell transplantation for a diffuse large B cell lymphoma. She was admitted to the ICU for neurological deterioration three months after transplantation, leukocyte count was 2.2×10^9/L and lymphocyte count 0.8×10^9/L. Two bronchoalveolar lavages were performed, seven days apart, which both contained RV-A24 with Cq values of 19and 18 respectively. Pulmonary symptoms started 15 days prior to the initial BAL. Galactomannan was positive in both BALs, no other viral or bacterial pathogens could be found. In total seven blood samples were tested, one tested positive, Cq 37, taken 13 days after the initial BAL. The patient died 16 days after the first BAL was performed. The last available blood sample before the patient died was rhinovirus negative. Shortly before death, progressive neurological deterioration, kidney insufficiency and Aspergillus pneumonia were observed. Autopsy was not performed.

Patient 4, a 48-year-old male with a kidney transplant was admitted at the ICU with respiratory insufficiency of unknown cause. The patient had 23.3×10^9/L leukocytes and 0.7×10^9/L lymphocytes at the time of the second BAL. Three BALs were performed, with RV Cq values of 24, 22and 17respectively. The second BAL, containing RV-B92, is used as index BAL in Fig. 1. The only pathogen consistently detected in all three BALs was rhinovirus. In total six blood samples were tested for rhinovirus, one tested positive Cq 36, taken 52 days after the first day of illness, being 16 days before the patients died of respiratory failure. Autopsy was not performed, but a lung biopsy, taken before the patient deceased, showed strongly disturbed lung architecture with few inflammatory infiltrate (no PCR performed). This was suggestive for a cryptogenic organizing pneumonia, possibly due to recurrent infections.

4.5. Rhinovirus viremia risk factors

In both the viremic and non-viremic groups the majority of patients had received allogeneic stem cell transplantation (Table 1). Almost all patients had clinically and radiologically signs of severe lower respiratory tract disease (cough, dyspnoea, radiological abnormalities).

No significant difference could be found between viremic and non-viremic patients with regard to underlying condition, symptoms, radiological findings or co-pathogens.

All patients with rhinovirus viremia died (4/4, 100%) as a consequence of respiratory problems during their hospital admission. Only five out of 23 (22%) rhinovirus viremia negative patients died during hospital admission, of which three died because of respiratory failure (all three had a co-infection with another virus). This difference in mortality was significant ($p = 0.007$, Fisher's exact).

5. Discussion

This study demonstrated the presence of rhinovirus viremia in four out of 27 adult patients with high loads in the respiratory tract. In addition, an association with higher mortality in the rhinovirus viremic patients was found. No other correlations could be found.

These results indicate that rhinovirus viremia may be a relevant negative prognostic factor given the significant association with fatal outcome. No other risk factors for viremia could be identified. In previous studies in children, rhinovirus viremia was associated with worse clinical signs and symptoms and was found in several fatal cases, also suggesting that viremia may be a predictor of poor prognosis [20–22,25–27]. Whether rhinovirus viremia would play a causative role in this poor prognosis, in the presence or absence of co-pathogens,

Table 1
Characteristics of rhinovirus viremia positive versus rhinovirus viremia negative patients.

	RV viremia positive (n = 4)	RV viremia negative (n = 23)	odds ratio	95% confidence interval		P* value
				lower	upper	
General						
Age, years, median (range)	60 (48–68)	56 (21–78)		−10.6	18.9	0.57
Male, no. (%)	2 (50)	15 (65)	0.53	0.06	4.5	0.62
Admission to hospital (%)	4 (100)	22 (96)				0.67
Conditions underlying						
Smoking	2 (2/3)	8 (8/17)	2.25	0.2	29.8	1
Pulmonary condition underlying	1 (1/4)	5 (5/22)	1.13	0.1	13.4	1
STx (%)	3 (75)	12 (52)	2.75	0.2	30.5	0.61
Allogeneic	3 (3/3)	11 (11/12)				1
NMA	2 (2/3)	7 (7/11)				1
Related	0 (0/3)	4 (4/11)				0.51
GVHD	0 (0/3)	6 (6/12)				0.23
Solid organ Tx (%)	1 (25)	5 (22)	1.2	0.1	14.2	1
Prednisone use (%)	1 (25)	12 (52)	0.31	0.03	3.4	0.60
Symptoms						
Fever	2 (2/3)	18 (18/23)	0.56	0.04	7.5	1
Coughing	3 (3/3)	14 (14/15)				1
Dyspnoea	3 (3/3)	21 (21/21)				
Laboratory findings						
CRP mg/L, mean (range)	115 (22–162)	131 (3–417)		−162.4	131.3	0.83
Leukocytes x10^9/L, mean (range)	7.3 (0.1–23.3)	7.7 (0.1–22.9)		−7.8	6.9	0.91
Lymphocytes x10^9/L, mean (range)	0.53 (0 − 0.83)	1.09 (0.01 − 6.16)		−2.2	1.0	0.47
Saturation%, without oxygen, mean(range)	96 (90–100)	94 (88–100)		−3.4	6.6	0.51
pH, mean (range)	7.45 (7.38 − 7.54)	7.42 (7.24 − 7.52)		−0.06	0.1	0.56
Radiography						
Abnormalities (%)	4 (100)	22 (96)				1
Ground glass	2 (2/3)	8 (8/16)	2	0.2	26.7	1
Consolidations (%)	4 (100)	18 (78)				0.56
Tree in bud	1 (1/3)	7 (7/16)	0.64	0.05	8.6	1
Microbiology						
Duration of respiratory disease to BAL, mean (range)	7 (1–15)	12 (2–36)		−14.8	5.5	0.36
Days post-Tx and BAL sampling date, mean (range)	343 (60–1034)	517 (17–4455)		−1300.6	951.8	0.75
Rhinovirus BAL Cq value, mean (range)	22.9 (19.4–24.5)	20.6 (15.5–24.9)		−0.6	5.1	0.12
Respiratory co-pathogens, viral, bacterial or fungal (%)	3 (75)	17 (74)	1.06	0.09	12.2	1
No. of blood samples available for RV testing per BAL, mean (range)	3.6 (2–6)	2.6 (1–6)		−0.8	2.9	0.26
Outcome						
Days admitted, mean (range)	35 (12–53)	24 (2–106)		−16.4	37.26	0.43
Deceased (%)	*4 (100)*	*5 (22)*				*0.01*

Abbreviations: RV: rhinovirus, STx: stem cell transplantation, BAL: bronchoalveolar lavage, Tx: transplantation, GVHD: Graft-versus-host-disease.
* Fisher's exact or independent T-test.

or whether it indicates end stage disease needs to be determined. Similarly, while we did not culture our blood samples we cannot deduce whether rhinovirus viremia is the result of leakage of viral RNA from the lungs, as may be suggested by the severity of illness and the high Cq values, or whether active replication takes place outside the respiratory tract. However, others have shown previously that rhinovirus could be cultured from the blood of children with severe rhinovirus infections [21,26]. Furthermore, in vitro, rhinovirus has been cultured on Hela cell suspension [31] suggestive of a broader cell tropism resembling enterovirus.

We were able to test an average of three-four blood samples per BAL sample. However, sufficient volumes of blood were not always available to enable testing with optimal sensitivity. In addition, a potential classification bias may have been introduced if more blood samples were drawn, stored and tested from more severely ill patients with high mortality risk compared to less severely ill patients. However, our comparison of the mean number of blood samples tested for RV per patient shows that this was comparable for the RV viremic patients and controls.

Patients with high viral loads in the BAL were selected for rhinovirus viremia testing. High viral loads in BAL fluid, with an arbitrary threshold of Cq < 25, were selected as it was expected these would show stronger correlations with viremia. Despite this potentially high-risk population only a minority of patients were viremic, with low viral loads. This percentage in the selected group of highly immunocompromised adults is comparable to the percentage of viremia described in immunocompetent children, while in immunocompromised children the rate of viremia may be considerably higher. Viremia in immunocompetent adults may occur even less frequently, although this could not be studied in our population. It must be noted that the low number of viremic patients reduces the power of the risk factor analysis. In children RV viremia has been shown to be associated with asthma [20,25], therefore adults with asthma or COPD may also have a higher risk of RV viremia. The effects of asthma or COPD could not be determined, given the low number of patients with underlying pulmonary conditions.

In children, the percentage of rhinovirus viremia positive cases has been reported to be higher on the day of admission compared to three days later [23,25]. In our adult patients this pattern could not be detected, potentially because blood samples were not drawn on a regular basis and the number of positive blood samples was low.

Genotyping of the rhinovirus viremia positive samples revealed two RV-A, two RV-B and no RV-C genotypes, though RV-C appeared to be the predominant species in viremic children. The percentage RV-C in children with viremia varies from 67% to 87% in studies with six and 30 viremic patients respectively [20,23]. Because the number of viremic patients is low and the local prevalence of the different rhinovirus species over the years is unknown, it cannot yet be concluded

whether the outcome of RV-C viremia is different between children and adults.

In conclusion, rhinovirus viremia may occur in adult patients, possibly less frequent compared to children, and appears to be associated with a higher mortality. The role of such viral markers may also be relevant if broader genotypic approaches (sequencing) will increasingly be used in the near future. The pathogenesis and risk factors of rhinovirus viremia need further investigation.

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None.

Competing interests

None declared.

Ethical approval

The review board declared to have no objections against the study.

Disclosures

Any part or whole of the manuscript has never been published.

Author's contributions

ALR, ECC and JJCV participated in the study design. ALR collected the data, and ALR, PAB, ECC, ACMK and JJCV analysed and interpreted the data. ALR wrote the first version of the manuscript. ECC, PAB, ACMK and JJCV contributed and revised the manuscript. All authors read and approved the final manuscript.

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