# 成人血液内科病棟におけるRSウイルスアウトブレイクの臨床的検討と遺伝子系統樹解析

# The clinical and phylogenetic investigation for a nosocomial outbreak of respiratory syncytial virus infection in an adult hemato-oncology unit

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*Shortened title: RSV outbreak in an adult hemato-oncology ward*

**Abstract**

*Statement of the problem*

Although many reports have already shown RSV outbreaks among hemato-oncology patients, genomic studies detecting similar RSV strains prior to an outbreak in the hospital are rare. In 2014, the University of the Ryukyus hospital hemato-oncology unit experienced, and successfully managed, a respiratory syncytial virus (RSV) nosocomial outbreak. During the outbreak investigation, genotyping and phylogenetic analysis was used to identify a potential source for the outbreak.

*Method of study*

Nasopharyngeal swabs were tested for RSV using three tests, 1) rapid antigen test (RAT), 2) reverse transcriptase polymerase chain reaction (PCR), or 3) quantitative PCR (RT-qPCR); a positive PCR reaction was considered a confirmed case of RSV. Phylogenetic analysis of the G protein was performed for outbreak and reference samples from non-outbreak periods of the same year.

*Results*

In total, twelve confirmed cases were identified, including eight hemato-oncology patients. Patient samples were collected weekly, until all confirmed

RSV cases returned RSV negative test results. Median time of suspected viral shedding was 16 days (n=5, range: 8-37 days). Sensitivity and specificity of the RAT compared with RT-qPCR were 30% and 91% (n=42). Phylogenetic analysis revealed nine genetically identical strains; eight occurring during the outbreak time period and one strain was detected one month prior.

*Conclusions*

A genetically similar RSV detected one month before is considered one potential source of this outbreak. As such, healthcare providers should always enforce standard precautions, especially in the hemato-oncology unit.

**Keywords**

Respiratory syncytial virus, outbreak, hemato-oncology patient, viral shedding, rapid antigen test, phylogenetic analysis

## Introduction

Respiratory syncytial virus (RSV) is a ubiquitous viral pathogen significantly impacting children and adults [Holman et al., 2003; Jain et al., 2015; Lee et al., 2013; Volling et al., 2014]. Although it is recognized as the most common cause of lower respiratory tract infection (LRTI) in children, this virus can also be problematic in immunocompromised patients, especially hemato-oncology patients [Branche and Falsey, 2015]. Indeed, recent reports portray this virus as the cause of severe, even life threatening, LRTI in immunocompromised adults, including hemato-oncology patients [Ariza-Heredia et al., 2012; Avetisyan et al., 2009; Chemaly et al., 2006; El Saleeby et al., 2008; Khanna et al., 2008; Kim et al., 2014; Pilie et al., 2015; Shah et al., 2013]. Furthermore, it is understood that RSV can be transmitted easily between hemato-oncology patients, resulting in outbreaks [Abdallah et al., 2003; Chu et al., 2014; Jensen et al., 2016; Kelly et al., 2016; Lehners et al., 2013; Mazzulli et al., 1999; Mendes et al., 2013; Singh et al., 2015; Taylor et al., 2001]. Although many reports of RSV outbreaks among hemato-oncology patients exist, few articles have detected similar RSV strains prior to outbreak conditions in the hospital using genomic analysis [Geis et al., 2013].

Between August and September 2014, an RSV outbreak occurred in a ward shared between the hemato-oncology and endocrinology departments of the University of the Ryukyus hospital in Okinawa, Japan. In the beginning, two hemato-oncology patients had PCR positive results. An outbreak was suspected soon after, because multiple patients and healthcare providers began experiencing acute respiratory symptoms. In total, eight patients and two providers were confirmed RSV positive. RSV type B was detected through PCR and the infection control team (ICT) communicated the outbreak conditions to all doctors in the hospital. Following the ICT's intervention procedures, no new cases were discovered. The outbreak was terminated after 17 days.

Here, an RSV outbreak in the hemato-oncology unit of our university hospital was experienced. Amidst the outbreak: the clinical features of RSV infected patients were investigated; the utility of rapid antigen tests (RAT) were evaluated; and genomic analysis to confirm the genetic identity of outbreak strains was conducted. Additionally, phylogenetic analysis of previously collected RSV samples provides a possible timeline of events.

## Materials and Methods

*Specimen collection and diagnostics*

Samples were collected from symptomatic patients and healthcare providers during the outbreak. Nasal swabs (bronchoalveolar lavage from one patient) were collected from the patients and medical staff, and tested with a RAT (ImmunoAce RSV, Towns, Japan). The residual liquid, following RAT completion, underwent nucleic acid extraction using a commercially available extraction kit (Ribospin™ vRD, GeneAll®, South Korea). Eluted samples were tested with the multiplex reverse transcriptase-PCR (RT-PCR) kit (Seeplex® RV15 OneStep ACE Detection, Seegene, South Korea) and multiplex quantitative RT-PCR (RT-qPCR) kit (Anyplex™ II RV16 Detection, Seegene, South Korea). Subjects with positive results for one of the two PCR tests were considered a confirmed case. Confirmed cases were followed until confirmation of negative results.

*Review of the medical records and ethics*

The medical records of all confirmed cases, excluding healthcare providers, were retrospectively reviewed with identifying information removed. All relevant clinical information was extracted and compiled. For the purposes of this paper, viral shedding was defined as the duration, in days, between the first

positive result and simultaneous negative results from both PCR tests. Probable onset dates were determined from the ICT interview with patients and attending doctors combined with medical record data. This study was reviewed and approved by the Clinical Research Ethics Committee of University of the Ryukyus (H27.8-9-844).

*Statistical analysis for agreement*

Agreement among the results obtained by the three diagnostics; RAT, RT-PCR, and RT-qPCR, was analyzed by the Fleiss κ test using IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp. The relative sensitivity and specificity of RAT were also calculated compared to RT-qPCR as a standard [Kim et al., 2013].

*Gene sequencing*

Amplification and sequencing of the G protein gene was performed for phylogenetic analysis. All RSV-B positive samples from the outbreak and non-outbreak periods of the same year collected in the hospital were included in the analysis. Nucleic acid amplifications were carried out with TaKaRa premix

Taq™ (TaKaRa, Japan). Reactions were performed at a final volume of 50 µl. The thermocycler was programmed; 94 °C for 5 minutes, 40 cycles at 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 60 seconds, followed by 72 °C for 5 minutes elongation. A primer set was designed using the National Center for Biotechnology Information's (NCBI) Primer-BLAST program (National Center for Biotechnology Information. Primer-BLAST. Accessed 9 June 2016 <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>.). The resulting primers (5'ACAAACCAAAGGCAGAACCTCTA3' and 5'GATGCTGTGGGTGTCTGTGT3') were based on the reference sequence NC_001803 for a final product length of 513 bp. Hokkaido System Science Co., Ltd performed the nucleotide sequencing, using the Sanger method. Samples were additionally sent to Osaka University for verification.

*Phylogenetic analysis*

Phylogenetic analysis of the in-hospital strains' G protein chromatograms was performed using MEGA (version 6.0; DNASTAR, Madison, WI) with the Neighbor-Joining tree method (NJM) using the reference sequence, NC_001803. An NCBI BLAST search provided the sequence data of closely related worldwide

150 strains from the GenBank database. A total of 101 closely matching sequences
151 were compared to the outbreak strains using NJM.
152
153 **Results**
154 Samples were collected from eighteen symptomatic patients and five
155 symptomatic healthcare providers in the ward. RSV was identified in ten patients
156 and two providers (table 1). Eight of the ten patients were hemato-oncology cases.
157 Death occurred in two patients with an advanced stage of hematological
158 malignancy. Mortality was due to the progression of their primary disease and not
159 related to RSV infection. Two patients with type 2 diabetes mellitus and two
160 healthcare staff also tested positive for RSV. All RSV positive cases were
161 considered mild, without severe symptomatology. Thus, no cases were treated
162 with anti-viral therapy.
163 Table 2 shows the comparison of patient characteristic between RSV
164 positive and negative cases. One patient, positive for rhinovirus but negative for
165 RSV, was excluded in this assessment. RSV positive patients were more likely to
166 share a room with another RSV positive patient (before cohorting), have chest
167 radiological findings, and present with cough. However, only the presence of

168 radiological findings was significantly different between the two groups in
169 univariate analysis.

170 Figure 1 shows the timeline of events. Almost all hemato-oncology
171 patients had respiratory symptoms regardless of probable onset date and PCR
172 positivity. Case 7 had respiratory symptoms prior to admission. Case 9 and 10,
173 diabetes patients, went out of hospital just before supposed onset. Adenovirus
174 was also detected from case 1 during follow up PCR testing. Viral shedding was
175 measured for only five hemato-oncology patients, due to patient discharge or
176 death, and the median length was sixteen days (range: 8-37 days).

177 The nucleotide sequence for G protein was successfully amplified for nine
178 patient samples within the outbreak timeline and seven patient samples from
179 previous RSV cases. The bootstrap consensus tree based on NJM shows that eight
180 outbreak strains were genetically identical, and one was slightly different (Figure
181 2). The strain named "Jul.25 inpatient" was obtained from an inpatient admitted
182 in an adjacent ward one month prior to the outbreak. This sample also proved to
183 be genetically identical to the other eight outbreak strains. The radial tree shows
184 the outbreak strains cluster within the Asia 2012-2014 sources (Figure 3).

185 Figure 4 shows a snapshot bed map of the ward where the outbreak

186 occurred on August 20th (date when first case was diagnosed). Phylogenetic
187 results and probable onset dates are also indicated. Confirmed cases, especially
188 genetically identical strains, were clustered on the north side of the ward. Some
189 patients had no association with RSV positive patients or healthcare providers
190 (i.e., cases 6, 9 and 10). Only patients with acute respiratory symptoms were
191 tested for RSV, despite contact with RSV positive healthcare providers.

192 During the study period, 46 nasal swabs were collected from 23 unique
193 individuals and 42 specimens were tested with all three tests; RAT, RT-PCR, and
194 RT-qPCR (Table 3). The κ score of RAT and RT-PCR, RAT and RT-qPCR and RT-PCR
195 and RT-qPCR were 0.313, 0.215 and 0.714, respectively. Relative sensitivity and
196 specificity of RAT were 30.0% and 90.9%, respectively. These rates were adjusted
197 to 27.8% and 93.3%, respectively, when limiting the population hemato-oncology
198 patients alone.

199

200 **Discussion**

201 Recently, there have been multiple reports of RSV outbreaks among
202 immunocompromised patients including hemato-oncology patients. Many of
203 which analyze the risk factors important to disease progression and fatal

204 outcomes (e.g. lymphocytopenia, hypogammaglobulinemia, hematopoietic stem
205 cell transplantation (HSCT), graft versus host disease after allogenic HSCT and
206 LRTI) [Ariza-Heredia et al., 2012; Avetisyan et al., 2009; Chemaly et al., 2006; El
207 Saleeby et al., 2008; Khanna et al., 2008; Kim et al., 2014; Lehners et al., 2013;
208 Pilie et al., 2015; Shah et al., 2013]. In this outbreak, most RSV-infected patients
209 experienced one or more of the risk factors listed above (Table 1). However, most
210 hemato-oncology cases did not experience severe infection from RSV. The two
211 mortal cases, in this study, were severe due to their underlying diseases, not due
212 to the influence of RSV. It is thought HSCT is the highest risk factor for severe
213 disease and fatal outcomes [Branche and Falsey, 2015; Hirsch et al., 2013;
214 Khanna et al., 2008; Shah et al., 2013]. It is possible that severe outcomes were
215 not experienced in this outbreak due to the limited number of HSCT cases.

216 It is plausible that known risk factors of fatal outcomes are also risk
217 factors for increased susceptibility. However, Table 2 shows no significant
218 differences in the patient backgrounds of RSV positive and negative cases. These
219 differences may have remained undetected due to the small sample size. RSV
220 positive cases were more likely to have radiological findings during outbreak
221 conditions. However, the chest radiological findings of RSV in

immunocompromised cases were faint and often required CT detection. CT findings have also been useful for indicating RSV infection in other studies with immunocompromised patients [Mayer et al., 2014].

The symptoms of RSV infection in healthy adult patients have been defined previously as cough and rhinorrhea (>80%), followed by high fever (60%) [Hall et al., 2001]. However, respiratory viruses, including RSV, among immunocompromised patients often occur without symptoms or with non-specific symptoms [Kuypers et al., 2009; Mikulska et al., 2014; Tomblyn et al., 2009]. Indeed, RSV positive cases in our outbreak frequently had cough and nasal discharge (Figure 1), but these symptoms cannot always be attributed to RSV infection and are frequently associated with the patient's primary diseases. As such, pinpointing when RSV symptoms began for the hemato-oncology patients and detecting the outbreak emergence pattern was problematic. In contrast, onset of symptoms for the two non-hemato-oncology patients was easily determined.

A recent systematic review showed RATs had enough sensitivity to detect RSV infection for infants (81% [95% CI; 78% to 84%]), however, the sensitivity of RATs for RSV in adults was decreased (29% [95% CI; 11% to 48%])

240 [Chartrand et al., 2015]. Despite this, studies evaluating RAT utility focusing on
241 immunocompromised adults are not well reported [Casiano-Colón et al., 2003;
242 Chartrand et al., 2015; Englund et al., 1996]. The utility of RAT in this study was
243 poor. Although RATs are convenient, doctors must recognize the high potential for
244 false-negative results, especially in adult populations, regardless of their immune
245 status.

246 The bootstrap consensus tree (Figure 2) showed only small differences
247 between outbreak and non-outbreak strains. One non-outbreak strain named as
248 "Jul.25 inpatient" was completely identical to the eight outbreak strains,
249 indicating, perhaps, that the malefactor strain responsible for the outbreak
250 originated and subsisted within the hospital one month prior to the recognition of
251 the outbreak when no other symptomatic cases were detected. Since the ward
252 has shared spaces, such as an elevator hall and conversation lounge, RSV
253 infection may have been transmitted throughout. The differences in the sequence
254 analysis do suggest case 10 was a strain of RSV from the community. In fact, this
255 patient was free to come and go from the hospital and RSV infection was common
256 among children in the region at that time.

257 Some reports show that genetically different RSVs can be detected during

one outbreak [Chu et al., 2014; Jensen et al., 2016; Mazzulli et al., 1999; Taylor et al., 2001], suggesting there may be multiple channels of RSV infection. The small differences observed among the genetic strains in this study may be representative of these multiple channels. The results from the radial tree indicate outbreak strains were similar to other sources, geographically, at that time (Figure 3). Therefore, it is likely that outbreak strains were initially contracted from one of the strains circulating in the community at that time [Chu et al., 2014; de-Paris et al., 2014; Geis et al., 2013].

According to recent reports, duration of viral shedding in community RSV infection ranges from 10-12 days [Munywoki et al., 2015; Walsh et al., 2013] when measured by PCR. Other studies report the duration is prolonged in immunocompromised patients, especially in HSCT patients, to 20-30 days [Avetisyan et al., 2009; Lehners et al., 2013]. A prolonged duration of viral shedding can be associated with severity of RSV infection [Munywoki et al., 2015; Walsh et al., 2013]. Prolonged viral shedding can also facilitate the outbreak of RSV infection and make it difficult to control nosocomial infection. In the present outbreak, the longest shedding case (case 8) had a history of allogenic transplant. However, the case presented with only mild symptoms and exhibited minimal

symptoms for the duration of viral shedding.

A trace-back investigation was performed using the phylogenetic analysis, onset dates and ward map. Case 10, as a genetically proven community strain, was excluded. Additionally, the cause of case 9 was unclear. Case 9 often went out of hospital and is another likely community infected case. Assuming case 8 was the first developed case in the ward, cases 2, 4 and 12 (an attending nurse) were infected following exposure to case 8. Sharing a room with a patient confirmed RSV positive is a considerable risk factor for RSV infection [Aichinger E et al., 2014]. Case 2 was eventually moved to a room already inhabited by case 3. Thus, case 3 was infected following exposure to case 2. The doctor attending case 3 was also infected to become case 11. There is some possibility that case 7 was infected following exposure to case 11, however, it is more likely that case 7 is a second community-infected case. Unfortunately, the patient sample for case 7 was not successfully amplified or analyzed. The aforementioned cases do not have a direct association with the rest of the confirmed cases, 1, 5, and 6. However, case 1, 5 and 6 also had strains determined to be identical to the outbreak strain, therefore some association must exist. It is possible these patients made contact in the shared community spaces, or that another patient, with undetected RSV infection,

294 acted as a transmitter without being noticed.

295 The ICT intervened using only three decisions: (1) cohorting of RSV
296 positive patients, (2) re-training of healthcare providers on standard precautions,
297 and (3) limiting admission to visitors and healthcare staff with respiratory
298 symptoms. A recent report supports the efficacy of cohorting to control an RSV
299 outbreak in a hemato-oncological unit [Aichinger E et al., 2014; Singh et al.,
300 2015]. Standard precautions (e.g. handwashing and alcohol hand rub, gloves,
301 masking) were strictly enforced among healthcare providers. Handwashing,
302 alcohol hand rub and masking were also suggested to patients and visitors of the
303 ward. RSV infections occur via droplet transmission and through physical contact,
304 therefore standard precautions are critical. Strict attention should also be given to
305 the symptomatology of healthcare personnel. According to a guideline to prevent
306 infectious complications in hemato-oncology units, health care providers having
307 respiratory symptoms should not be allowed to work because they will become a
308 potential community transmitter [Kelly et al., 2016; Tomblyn et al., 2009]. As
309 such, symptomatic healthcare personnel were granted an absence from work until
310 symptoms dissipated. The admission of visitors was also limited to the highest
311 possible extent. During the outbreak, only necessary visits were suggested;

whereas, prior to the outbreak, visitors were advised to stay home only if they were experiencing fever and/or any respiratory symptoms, or if anyone in the household was experiencing these symptoms. Respiratory viruses, not only RSV, remain ever present [Kelly et al., 2016; Kuypers et al., 2009]. Thus, limiting the admission of symptomatic workers and all visitors, with or without symptoms, will prevent additional pathogens from entering the ward. Following the interventions instituted by the ICT, the outbreak was quickly contained and terminated, regular trainings for standard precautions has been instituted and followed consistently, and clinicians no longer rely on negative RAT results alone for adult patients.

This study has certain limitations. First, this is retrospective study, therefore patient information was limited and symptoms and onset dates were not determined with a standardized protocol. Second, due to the small sample size, the potential risk factors of RSV infection, LRTI progression and mortality in adult hemato-oncology patients could not be determined.

In summary, community RSV strains can easily and repetitively invade the hospital and cause prolonged infection. Routine awareness of infection control is critical. Phylogenetic analysis was helpful to perform a trace-back investigation. This study demonstrates a successfully contained outbreak and reiterates that

healthcare providers should always follow standard precautions. It also suggests respiratory symptoms should be investigated with PCR, especially when treating immunocompromised patients.

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**Conflict of Interest**

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497 **Figure legends**

498 *Figure 1: Viral shedding, related symptoms and onset dates with time line*

499 Medical records were retrospectively reviewed. Length of viral shedding was
500 assessed in 5 hemato-oncology patients (Case 1, 2, 4, 8, and 9)

501

502 *Figure 2: Phylogenetic analysis (bootstrap tree)*

503 Phylogenetic analysis of the G protein using a Neighbor-Joining method was
504 performed. The reference sequence was obtained from GenBank, under accession
505 number NC_001803. Sequence data of Case 1 (Bronchoalveolar lavage), 3, 5, 6
506 and 10 were sequenced by Hokkaido, and Case 1 (Bronchial lavage), 2, 4 and 12
507 were sequenced by Osaka University.

508

509 *Figure 3: Phylogenetic analysis (radial tree)*

510 Phylogenetic analysis of the G protein using a Neighbor-Joining method was
511 performed. The radial tree shows the outbreak strains cluster within the Asia
512 2012-2014 highlighted region, indicating these outbreak strains were not unusual
513 in the geographic area at that time.

514

515 *Figure 4: Bed map with onset dates*

516 The map of Aug.20, date of case 1 diagnosed.