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Clinical Relevance of Multiple Respiratory Virus Detection in Adult Patients with Acute Respiratory Illness

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Because increasing numbers of nasopharyngeal swab specimens from adult patients with acute respiratory illness (ARI) are being tested by respiratory virus (RV) multiplex reverse transcriptase PCR (RVM-RT-PCR), multiple RV detection (MRVD) is being encountered more frequently. However, the clinical relevance of MRVD in adult patients has rarely been evaluated. The clinical characteristics of hospitalized adult patients with ARI and MRVD by RVM-RT-PCR tests were compared to those of patients with single RV detection (SRVD) during a single year at a tertiary care center. MRVD was observed in 26 of the 190 adult patients (13.7%). The patients with MRVD had a higher incidence of chronic lung disease than the patients with SRVD (34.6% versus 15.9%, crude odds ratio [OR] = 2.81, 95% confidence interval [CI] = 1.13 to 6.98, $P = 0.03$). Although the former were more likely than the latter to receive mechanical ventilation (19.2% versus 6.7%, crude OR = 3.31, 95% CI = 1.05 to 10.47, $P = 0.049$), the length of hospital stay (median, 7 versus 6.5 days; $P = 0.66$), and the in-hospital mortality rate (7.7% versus 4.3%, crude OR = 1.87, 95% CI = 0.37 to 9.53, $P = 0.35$) were not different between the two groups. In multivariate analysis, chronic lung disease was associated with MRVD (adjusted OR = 3.08, 95% CI = 1.12 to 8.46, $P = 0.03$). In summary, it was not uncommon to encounter adult patients with ARI and MRVD by RVM-RT-PCR tests of nasopharyngeal swab specimens. MRVD was associated with chronic lung disease rather than the severity of the ARI.

The respiratory virus (RV) multiplex reverse transcriptase PCR (RVM-RT-PCR) test has recently been used in clinical practice for the detection of RV (1). It allows the fast and simultaneous detection of a large number of RVs with a higher sensitivity than existing RV detection tests, such as viral culture or serological tests (1–3). With the recent increase in the number of patients who receive the RVM-RT-PCR test because of acute respiratory illness (ARI), we often encounter patients in whom more than one RV is detected by the test, a laboratory finding of multiple RV detection (MRVD). Many researchers have investigated the clinical relevance of RV coinfection using various viral diagnostic methods; this has primarily been investigated in pediatric patients but has also been investigated in patients of all ages (4–15). However, there have been few studies of the RVM-RT-PCR test only in adults (16–18). To investigate the clinical relevance of MRVD in adults with ARI, we compared the clinical characteristics of hospitalized adult patients with ARI and MRVD by the RVM-RT-PCR test of nasopharyngeal swab specimens with those of patients with a single RV detection (SRVD) during a single year at a tertiary care center.

MATERIALS AND METHODS

Patient selection and data collection. This study was performed at the Chung-Ang University Hospital, an 850-bed tertiary care teaching hospital in Seoul, Republic of Korea. We identified all adult patients (ages, ≥16 years) who had been admitted to our hospital, had received a RVM-RT-PCR test, and had a positive test result between 1 April 2013 and 10 March 2014. Electronic medical records and chest radiographs of these patients were reviewed. Patient demographics, the presence of pathogens other than RV, the presence of underlying diseases or conditions, the presence of respiratory symptoms, the acute physiology and chronic health evaluation II (APACHE II) score, a history of mechanical ventilation, the use of vasoconstrictive agents, a history of supplemental oxygen therapy, admission to the intensive care unit (ICU), and clinical outcomes were investigated. This study was approved by the hospital's institutional review board.

Definitions. ARI was defined as the development of at least one of the following respiratory symptoms within 2 weeks before undergoing the RVM-RT-PCR test: cough, sputum production, rhinorrhea, sore throat, and dyspnea. Pneumonia was defined as the presence of a new or progressive infiltrate on a chest radiograph plus three or more of the following symptoms or signs: fever, cough, sputum production, dyspnea, hemoptysis, and an attending physician's diagnosis of pneumonia (19). A patient was considered to have a community-acquired ARI if he or she had had an ARI within 2 days after admission and had undergone RVM-RT-PCR testing for the episode, to have a hospital-acquired ARI if he or she had had an ARI after 2 days of admission, and to have a health care-associated ARI if he or she had had a community-acquired ARI and at least one of the following: admission to an acute care hospital for at least 2 days within the 90 days prior to the infection, residence in a nursing home or long-term-care facility, receipt of intravenous antibiotic therapy within a month prior to the infection, and regular attendance at a hemodialysis clinic. Patients who had chronic obstructive pulmonary disease (COPD), interstitial lung disease, and a lung destroyed by tuberculosis were considered to have chronic lung disease. The diagnosis of a lung destroyed by tuberculosis was based on the presence of present or past tuberculosis with a

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finding of parenchymal destruction caused by tuberculosis, as verified by coexamination of chest radiographs by radiologists (20).

Diagnostic methods. During the study period, nasopharyngeal swab specimens obtained with flocked swabs were submitted in universal transport medium (Copan, Brescia, Italy) for testing for 16 types of RV.

Nucleic acids were extracted from 300-μl specimens using a Viral Gene-spin viral DNA/RNA extraction kit (iNtRON Biotechnology, Gyeonggido, Republic of Korea). cDNA was synthesized from the extracted RNA with a cDNA synthesis premix (Seegene, Seoul, Republic of Korea) and a GeneAmp PCR system 9700 (Applied Biomaterials, CA, USA).

Testing was performed to detect 16 RVs, 14 RNA viruses and 2 DNA viruses: adenovirus (ADV), influenza virus (FLU) type A (FLU-A), FLU-B, respiratory syncytial virus type A (RSV-A), RSV-B, parainfluenza virus 1 (PIV-1), PIV-2, PIV-3, PIV-4, human rhinovirus (HRV), human metapneumovirus (HMPV), human enterovirus (HEV), human coronavirus (HCoV) 229E, HCoV NL63, HCoV OC43, and human bocavirus (HBoV). An internal control was added to each specimen to check the entire process from nucleic acid extraction to PCR, according to the manufacturer's instructions (Seegene, Seoul, Republic of Korea). An Anyplex II RV 16 detection kit (Seegene, Seoul, Republic of Korea) was used to detect the 16 RVs according to the manufacturer's instructions. Briefly, the assay was conducted in a final volume of 20 μl containing 8 μl of cDNA, 4 μl of 5× RV primer, 4 μl of 8-methoxypsoralen, and 4 μl of 5× master mix with a CFX96 real-time PCR detection system (Bio-Rad, CA, USA).

Statistical analyses. We compared the patients with MRVD with the patients with SRVD primarily in terms of clinical outcomes. Other clinical characteristics and the identified RVs were also compared between the two groups. Statistical analyses were performed using SPSS software (version 18.0; SPSS, Chicago, IL, USA). Continuous variables were compared using Student's *t* test or the Mann-Whitney U test. Categorical variables were compared using a χ^2 test or Fisher's exact test. A *P* value of <0.05 was considered significant. Variables which had ≥5 events per variable and had *P* values of <0.1 in the univariate analysis were included in the logistic regression analysis, which was performed by the enter method.

RESULTS

During the study period, RVM-RT-PCR tests were performed on specimens from 593 adult patients, among whom 223 (39.1%) had positive test results. Of these, 23 did not have ARI, 6 had no information on the amount of time that had elapsed from the time of onset of symptoms to the time of receipt of the RVM-RT-PCR test, and 4 were duplicates. After excluding these 33 patients, 190 adults were included in the final study analysis. MRVD was observed in 26 of these 190 patients (13.7%). The rate of MRVD during flu season (April 2013 and January to March 2014) did not differ from that during non-flu season (14.5% versus 10.5%, $P = 0.53$).

More than 50% of the study patients were female ($n = 111$, 58.4%), and the mean age was 62.7 years (standard deviation, 19.63 years). The majority of patients had community-acquired infections ($n = 133$, 70.0%), followed by health care-associated infections ($n = 33$, 17.4%) and hospital-acquired infections ($n = 24$, 12.6%). The most common underlying disease was diabetes mellitus ($n = 50$, 26.3%), followed by chronic lung disease ($n = 35$, 18.4%), bronchial asthma ($n = 25$, 13.2%), solid tumor ($n = 20$, 10.5%), and chronic kidney disease ($n = 16$, 8.4%). Fifty percent of the study population ($n = 95$) had pneumonia, and 34 (17.9%) were admitted to the ICU. In-hospital mortality was observed in 9 patients (4.7%).

The baseline characteristics and symptoms of the patients and the initial severity of the infection in patients with MRVD were compared to those in patients with SRVD (Table 1). There were no differences in baseline characteristics between the two groups, except that the patients with MRVD had a higher incidence of chronic lung disease than the patients with SRVD (34.6% versus 15.9%, crude odds ratio [OR] = 2.81, 95% confidence interval [CI] = 1.13 to 6.98, $P = 0.03$). The patients with MRVD received mechanical ventilation more often than the patients with SRVD (19.2% versus 6.7%, crude OR = 3.31, 95% CI = 1.05 to 10.47, $P = 0.049$) and tended to have a higher rate of ICU admission (30.8% versus 15.9%, crude OR = 2.36, 95% CI = 0.93 to 5.99, $P = 0.09$). The treatments and outcomes of the patients with MRVD were also compared to those of the patients with SRVD (Table 1). The clinical outcomes of the patients with MRVD did not differ from those of the patients with SRVD. In a multivariate model including age, sex, APACHE II score, chronic lung disease, mechanical ventilation, and ICU admission, only chronic lung disease was associated with MRVD (adjusted OR = 3.08, 95% CI = 1.12 to 8.46, $P = 0.03$) (Table 2). In the subgroup of patients with pneumonia ($n = 95$), the MRVD group tended to have a higher incidence of mechanical ventilation (31.3% versus 11.4%, crude OR = 3.54, 95% CI = 1.00 to 12.52, $P = 0.06$) and ICU admission (50.0% versus 27.8%, crude OR = 2.59, 95% CI = 0.87 to 7.76, $P = 0.08$) than the SRVD group.

The most commonly detected RV was FLU ($n = 110$, 57.9%; for FLU-A, $n = 80$ [42.1%]; for FLU-B, $n = 31$ [16.3%]), followed by HRV ($n = 31$, 16.3%), ADV ($n = 20$, 10.5%), HCoV ($n = 18$, 9.5%; for HCoV 229E, $n = 10$ [5.3%]; for HCoV OC43, $n = 8$ [4.2%]; for HCoV NL63, $n = 2$ [1.1%]), HMPV ($n = 15$, 7.9%), RSV ($n = 8$, 4.2%; for RSV-A, $n = 1$ [0.5%]; for RSV-B, $n = 7$ [3.7%]), PIV ($n = 7$, 3.7%; for PIV-1, $n = 1$ [0.5%]; for PIV-2, $n = 4$ [2.1%]; for PIV-3, $n = 1$ [0.5%]; for PIV-4, $n = 1$ [0.5%]), HEV ($n = 5$, 2.6%), and HBoV ($n = 1$, 0.5%). The distribution of the 16 RVs in patients with MRVD and those with SRVD is presented in Table 3. HRV, HCoV 229E, HEV, and ADV were more commonly observed in patients with MRVD than in those with SRVD. HCoV OC43 tended to be more common in the MRVD group than in the SRVD group. In the subgroup with pneumonia, the patients with MRVD had higher rates of infection with HRV (43.8% versus 13.9%, crude OR = 4.26, 95% CI = 1.71 to 10.61, $P = 0.01$), HCoV 229E (25.0% versus 2.5%, crude OR = 36.00, 95% CI = 7.09 to 182.68, $P = 0.007$), HEV (18.8% versus none, $P = 0.004$), and ADV (37.5% versus 10.1%, crude OR = 5.63, 95% CI = 2.03 to 15.60, $P = 0.01$) than the patients with SRVD.

We investigated the clinical impact of individual RVs in the 35 patients with MRVD (26 from the present study and 9 from our previous study [21]) by comparing the frequencies of chronic lung disease, mechanical ventilation, ICU admission, and in-hospital mortality between patients with MRVD with and without any individual RV (Table 4). Chronic lung disease tended to be less common in patients with MRVD with FLU infection than in those without FLU infection (16.7% versus 47.1%, $P = 0.05$). Patients with MRVD with HCoV OC43 infection tended to have a higher rate of in-hospital mortality than those without (50.0% versus 9.7%, $P = 0.09$).

DISCUSSION

MRVD was detected in 13.7% of hospitalized adult patients with RVM-RT-PCR-positive ARI. Patients with MRVD had higher rates of chronic lung disease and mechanical ventilation than patients with SRVD. Although the patients with MRVD tended to have higher rates of ICU admission than the patients with SRVD,

TABLE 1 Univariate analysis of association between individual clinical characteristics and MRVD and comparison of treatment and clinical outcomes between MRVD and SRVD groups[a]

Characteristic	Patients with MRVD ($n = 26$)	Patients with SRVD ($n = 164$)	Crude OR	95% CI	*P* value
Male sex	10 (38.5)	69 (42.1)	0.86	0.37–2.01	0.73
Median (range) age (yr)	68 (16–94)	67 (16–94)			0.85
Age group (yr)					0.89
16–39	3 (11.5)	28 (17.1)			
40–64	7 (26.9)	43 (26.2)			
65–79	11 (42.3)	60 (36.6)			
≥80	5 (19.2)	33 (20.1)			
Route of acquisition					0.13
Community acquired	22 (84.6)	111 (67.7)			
Health care associated	1 (3.8)	32 (19.5)			
Hospital acquired	3 (11.5)	21 (12.8)			
Detection of pathogens other than RV	4 (15.4)	25 (15.2)	1.01	0.32–3.18	1.00
Underlying diseases or conditions					
Diabetes mellitus	7 (26.9)	43 (26.2)	1.04	0.41–2.64	0.94
Chronic lung disease	9 (34.6)	26 (15.9)	2.81	1.13–6.98	0.03
Bronchial asthma	2 (7.7)	23 (14.0)	0.51	0.11–2.31	0.54
Solid tumor	2 (7.7)	18 (11.0)	0.68	0.15–3.10	1.00
Hematologic malignancy	1 (3.8)	6 (3.7)	1.05	0.12–9.12	1.00
Cerebrovascular disease	2 (7.7)	15 (9.1)	0.83	0.18–3.85	1.00
Chronic kidney disease	1 (3.8)	15 (9.1)	0.40	0.05–3.14	0.70
Congestive heart failure	1 (3.8)	7 (4.3)	0.90	0.11–7.61	1.00
Absolute neutrophil count of <500/μl[b]	1 (3.8)	4 (2.4)	1.60	0.18–14.90	0.52
Immunosuppressive agent use[c]	1 (3.8)	7 (4.3)	0.90	0.11–7.61	1.00
Chemotherapy[c]	1 (3.8)	5 (3.0)	1.27	0.14–11.34	0.59
Surgery[c]	0	3 (1.8)			1.00
Pneumonia as type of ARI	16 (61.5)	79 (48.2)	1.72	0.74–4.02	0.20
Median (range) time (days) from onset of symptoms to PCR test	3 (0–10)	3 (0–14)			0.23
Initial clinical symptoms					
Cough	22 (84.6)	119 (73.0)	2.03	0.66–6.23	0.21
Sputum production	21 (80.8)	110 (67.1)	2.06	0.74–5.77	0.16
Rhinorrhea	2 (7.7)	36 (22.0)	0.30	0.07–1.31	0.09
Sore throat	1 (3.8)	23 (14.0)	0.25	0.03–1.90	0.21
Dyspnea	9 (34.6)	55 (33.5)	1.05	0.44–2.51	0.91
Fever	15 (57.7)	119 (72.6)	0.52	0.22–1.21	0.12
Myalgia	6 (23.1)	26 (15.9)	1.59	0.58–4.34	0.40
Initial clinical severity					
Median (range) APACHE II score[d]	9.5 (3–38)	9 (0–30)			0.68
Mechanical ventilation[e]	5 (19.2)	11 (6.7)	3.31	1.05–10.47	0.049
Vasopressor use[e]	1 (3.8)	11 (6.7)	0.56	0.07–4.50	1.00
Supplemental oxygen requirement[e]	12 (46.2)	73 (44.5)	1.07	0.47–2.45	0.88
Admission to ICU[e]	8 (30.8)	26 (15.9)	2.36	0.93–5.99	0.09
Treatment					
Antiviral therapy	12 (46.2)	75 (45.7)	1.02	0.44–2.33	0.97
Antimicrobial therapy	23 (88.5)	139 (85.3)	1.32	0.37–4.76	1.00
Outcomes					
In-hospital mortality	2 (7.7)	7 (4.3)	1.87	0.37–9.53	0.35
14-day mortality	2/22 (9.1)	3/140 (2.1)	4.57	0.72–29.03	0.14
28-day mortality	2/18 (11.1)	6/130 (4.6)	2.58	0.48–13.90	0.25
Median (range) length of hospital stay (days)	7 (1–60)	6.5 (1–149)			0.66

[a] Unless otherwise noted, data are presented as the number (percent) of cases.
[b] Within a week before RVM-RT-PCR testing.
[c] Within a month before RVM-RT-PCR testing.
[d] Within 24 h after RVM-RT-PCR testing.
[e] Within a week after RVM-RT-PCR testing.

the clinical outcomes were not different between the two groups. Chronic lung disease was independently associated with MRVD.

The rate of MRVD observed in our study (13.7%) is consistent with the rates reported in the few previous studies performed only in adult patients: 12.0% (21/181) of Spanish adults during the 2009 influenza pandemic (18), 14.6% (30/220) of Chinese adults with ARI (16), and 12.5% (9/72) of South Korean adults requiring ICU admission for severe pneumonia (17). MRVD is known to

TABLE 2 Multivariate analysis of association between clinical characteristics and MRVD

Characteristic	Adjusted OR	95% CI	*P* value
Age	1.01	0.98–1.04	0.57
Female sex	1.63	0.65–4.11	0.30
APACHE II score[a]	1.00	0.91–1.10	0.99
Chronic lung disease	3.08	1.12–8.46	0.03
Mechanical ventilation[b]	2.27	0.38–13.41	0.37
Admission to ICU[b]	1.57	0.35–7.06	0.56

[a] Within 24 h after RVM-RT-PCR testing.
[b] Within a week after RVM-RT-PCR testing.

occur at a lower frequency in adult patients than in pediatric patients (7, 14, 15). While MRVD was observed in less than 15% of adult patients in previous studies (16–18), consistent with the findings of our current study, the rate was >15% in the majority (11/16) of studies of pediatric patients less than 6 years of age (14). Also, in a recent study including all age groups, patients aged 5 to 18 years and those younger than age 5 years had odds ratios 2.3 times and 3.2 times higher, respectively, than those aged more than 18 years (7). It has also been reported that the rate of MRVD has increased with more sensitive viral diagnostics (4). Compared to the rate of 5.0% in a study including all age groups that was performed 2 decades ago and that primarily used viral culture and serological tests (4), the rate of detection in recent PCR studies (of all age groups) ranges from 7.4 to 23.3% (5–10, 14, 15). Although we may encounter MRVD less frequently in adult patients than in pediatric ones, a rate of 13.7% is not negligible. Moreover, in the near future MRVD may be detected more frequently using newer viral diagnostics. Further evaluation of the incidence of MRVD and its clinical relevance is required.

The clinical relevance of MRVD is an unresolved issue. Although we are familiar with the notion that multiple viruses cause more severe illness than a single virus, several studies showed contradictory findings (4–18). Recent meta-analyses did not find more severe illnesses in patients with MRVD than those with SRVD (14, 15). However, the authors did not provide any conclusive remarks on this issue; rather, they pointed out that comparative analysis of clinical severity between MRVD and SVRD groups was not adjusted for important baseline characteristics, such as underlying diseases/conditions, in the majority of previous studies. Moreover, MRVD data on adult patients are extremely rare, and several studies including all age groups did not specify the clinical relevance of MRVD specifically in adult patients (5–10). Only two previous studies were performed exclusively in adult patients (17, 18). One, which focused on severe pneumonia requiring ICU admission, included only 9 patients with MRVD and did not present a comparison of clinical severity between MRVD and SRVD groups (17). The other, which focused on the 2009 influenza pandemic, showed a longer hospital stay in the MRVD group than in the SRVD group (mean lengths of stay, 2.3 versus 0.6 days; $P = 0.0019$), despite similar baseline characteristics, initial severity, and anti-influenza treatment between the two groups (18). However, because the study was performed during the pandemic period, the patients were relatively young (mean age, 39 years), rarely had chronic disease (diabetes, 12%; COPD, 6%), and were admitted infrequently to general wards (21%) and rarely to the ICU (only one patient). Considering that our patients were older (mean age, 63 years) and frequently chronically ill (diabetes, 26%; chronic lung disease, 18%), that all our patients were admitted, and that 18% of them received ICU care, their conclusions seem to be confined to the 2009 pandemic situation. Moreover, the length of hospital stay, the main outcome variable in the study, may not be an appropriate indicator of severe illness of the whole study population, because only a fifth of the patients were hospitalized. Thus, compared to previous studies on the issue, our study may provide more information, especially for adult patients.

Although a history of mechanical ventilation and admission to the ICU—indicators of clinical severity—were more common in the MRVD group, the clinical outcomes for the MRVD group did not differ from those for the SRVD group. Furthermore, in multivariate analysis, MRVD was not associated with mechanical ventilation or admission to the ICU. MRVD was associated only with chronic lung disease. Patients with structural lung disease, such as

TABLE 3 Comparison of RVs detected between hospitalized adult patients with ARI and MRVD from RVM-RT-PCR testing of nasopharyngeal swab specimens and those with SRVD

RV detected	No. (%) of patients with: MRVD ($n = 26$)	SRVD ($n = 164$)	Crude OR	95% CI	*P* value
Influenza A virus	12 (46.2)	68 (41.5)	1.21	0.53–2.78	0.65
Influenza B virus	3 (11.5)	28 (17.1)	0.63	0.18–2.26	0.58
Rhinovirus	10 (38.5)	21 (12.8)	4.26	1.71–10.61	0.003
Parainfluenza virus 1	0	1 (0.6)			1.00
Parainfluenza virus 2	0	1 (0.6)			1.00
Parainfluenza virus 3	0	4 (2.4)			1.00
Parainfluenza virus 4	1 (3.8)	0			0.14
Respiratory syncytial virus type A	0	1 (0.6)			1.00
Respiratory syncytial virus type B	1 (3.8)	6 (3.7)	1.05	0.12–9.12	1.00
Coronavirus NL63	1 (3.8)	1 (0.6)	6.52	0.40–107.61	0.26
Coronavirus 229E	8 (30.8)	2 (1.2)	36.00	7.09–182.68	<0.001
Coronavirus OC43	3 (11.5)	5 (3.0)	4.15	0.93–18.53	0.08
Human metapneumovirus	2 (7.7)	13 (7.9)	0.97	0.21–4.56	1.00
Human enterovirus	5 (19.2)	0			<0.001
Adenovirus	8 (30.8)	12 (7.3)	5.63	2.03–15.60	0.002
Human bocavirus	0	1 (0.6)			1.00

TABLE 4 Comparison of frequency of chronic lung disease, mechanical ventilation, admission to ICU, and in-hospital mortality between patients with and those without any individual RV among 35 hospitalized adult patients with an ARI and MRVD[a]

Characteristic	FLU included in MRVD			HRV included in MRVD			ADV included in MRVD			CoV 229E included in MRVD			HEV included in MRVD			CoV OC43 included in MRVD		
	No. (%) of cases			No. (%) of cases			No. (%) of cases			No. (%) of cases			No. (%) of cases			No. (%) of cases		
	Yes ($n = 18$)	No ($n = 17$)	*P*	Yes ($n = 13$)	No ($n = 22$)	*P*	Yes ($n = 9$)	No ($n = 26$)	*P*	Yes ($n = 8$)	No ($n = 27$)	*P*	Yes ($n = 9$)	No ($n = 26$)	*P*	Yes ($n = 4$)	No ($n = 31$)	*P*
CLD	3 (16.7)	8 (47.1)	0.05	3 (23.1)	8 (36.4)	0.48	3 (33.3)	8 (30.8)	1.00	4 (50.0)	7 (25.9)	0.23	4 (44.4)	7 (26.9)	0.42	2 (50.0)	9 (29.0)	0.57
MV	3 (16.7)	5 (29.4)	0.44	4 (30.8)	4 (18.2)	0.43	1 (11.1)	7 (26.9)	0.65	2 (25.0)	6 (22.2)	1.00	3 (33.3)	5 (19.2)	0.40	2 (50.0)	6 (19.4)	0.22
ICU	4 (22.2)	7 (41.2)	0.23	6 (46.2)	5 (22.7)	0.26	0	5 (19.2)	0.30	3 (37.5)	8 (29.6)	0.69	6 (33.3)	8 (30.8)	1.00	2 (50.0)	9 (29.0)	0.57
Death	2 (11.1)	3 (17.6)	0.66	3 (23.1)	2 (9.1)	0.34	3 (33.3)	8 (30.8)	1.00	0	5 (18.5)	0.31	2 (22.2)	3 (11.5)	0.59	2 (50.0)	3 (9.7)	0.09

[a] FLU, influenza virus; HRV, rhinovirus; ADV, adenovirus; CoV, coronavirus; HEV, human enterovirus; CLD, chronic lung disease; MV, mechanical ventilation; ICU, admission to the intensive care unit; Death, in-hospital mortality.

COPD, are known to be vulnerable to RV infections through impaired local immunity (22, 23). Thus, our data suggest that MRVD is a marker of an impaired respiratory immune system rather than the clinical severity of the disease. Various scenarios may be possible in patients with a finding of MRVD, for example, simultaneous infection with multiple RVs, initial infection with one RV overlapping with subsequent infection with another RV, or an already passed infection with one RV combined with a new infection with another RV. Irrespective of the condition, MRVD may suggest that the patient has repeated RV infections within a short time due to an impaired respiratory immune system. Further evaluation of MRVD and its association with chronic lung disease may be needed in adult patients with ARI.

In our study, HRV, HEV, ADV, and HCoV 229E were more commonly found in the patients with MRVD than in the patients with SRVD. These RVs were also frequently found in other studies of MRVD in adults. In the study of the 2009 influenza pandemic (18), picornaviruses (HRV and HEV) were most commonly combined with influenza viruses (19 of 21 cases). Among the 30 patients with MRVD in the other study (16), the following pairs were detected the most frequently: picornavirus and FLU-A ($n = 8$), picornavirus and HCoV 229E ($n = 6$), FLU-A and HCoV 229E ($n = 5$), and picornavirus and ADV ($n = 5$). In studies including all age groups, the RVs mentioned above were also commonly found in patients with MRVD (5–10). RSV, which has frequently been identified in MRVD studies including pediatric patients (11–15), seems to be infrequent in MRVD in adult patients.

The possible role of individual RVs in MRVD is unknown and another subject of interest. For example, some RV infections may worsen or lessen the severity of another RV infection. Thus, we evaluated the clinical impact of several individual RVs in patients with MRVD (Table 4). Esper et al. suggested that coinfection with HRV tended to have a lower clinical severity than coinfection without HRV (6); however, we did not find such results. Several studies performed in pediatric patients suggested that RSV increased the severity of coinfection of another RV (12, 13, 24). However, the impact of RSV could not be evaluated in our study because of the small number of RSV infections in adult patients with MRVD. Further evaluation of the more frequently found RVs in MRVD may be required to elucidate the complex pathogenesis and interactions of these RVs.

There were several important limitations in this study. First, the indication to request a RVM-RT-PCR test was based on the physician's assessment of each patient. Thus, some patients with appropriate symptoms might not have been screened. Second, we did not perform quantitative analysis of the viral load of each RV. Such an analysis might help to elucidate the interactions of individual RVs in MRVD. Third, RVM-RT-PCR tests were not performed with lower respiratory tract specimens, such as bronchoalveolar lavage fluid specimens, in cases of pneumonia. Unrevealed pathogenic RVs or other microorganisms in the lower respiratory tract might affect the analysis of our data. However, other studies on the issue had similar limitations (4–13, 16, 18). Moreover, clinically detected pathogens other than RVs were similarly distributed among the patients with MRVD and SRVD in our study.

In conclusion, MRVD was found in 13.7% of nasopharyngeal swab samples of adult patients with RVM-RT-PCR-positive ARI and was associated with chronic lung disease rather than clinical severity.

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