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Replicative virus shedding in the respiratory tract of the patients with Middle East respiratory syndrome coronavirus infection

Short communications

Running Head: Detection of MERS-CoV subgenomic mRNA

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Highlights

- Detection of MERS-CoV subgenomic mRNAs indicates the virus is replicative.
- Subgenomic mRNA was detected in lower respiratory tract specimens up to 4 weeks after symptoms developed.
- In upper respiratory tract specimens, detection of subgenomic mRNA and genomic RNA did not correlate.

Abstract

Background: Information on the duration of replicative Middle East respiratory syndrome coronavirus (MERS-CoV) shedding is important for infection control. Detection of MERS-CoV subgenomic mRNAs indicates the virus is replicative. We examined the duration for detecting MERS-CoV subgenomic mRNA compared with genomic RNA in diverse respiratory specimens

Methods: From upper and lower respiratory samples of 17 MERS-CoV-infected patients,
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MERS-CoV subgenomic mRNA was detected by reverse transcription polymerase chain reaction (RT-PCR) and MERS-CoV genomic RNA was done by real time RT-PCR.

Results: In sputum or transtracheal aspirate, subgenomic mRNA was detected up to 4 weeks after symptoms developed, which correlated with the detection of genomic RNA. In oropharyngeal or nasopharyngeal swab specimens, detection of subgenomic mRNA and genomic RNA did not correlate.

Conclusions: These findings suggest that MERS-CoV does not replicate well in the upper respiratory tract.

Keywords: Coronavirus Infections; RNA, Messenger; Respiratory System

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) genomic RNA can persist for more than 1 month in respiratory specimens.¹ However, MERS-CoV RNA detection may overestimate the duration of shedding of replicative virus. Coronaviruses (CoVs) have a unique mechanism of discontinuous transcription with the synthesis of subgenomic mRNAs.² The MERS-CoV has at least seven distinct subgenomic mRNA species and detection of these indicates the virus is replicative.³ The objectives of this study was to examine the duration for detecting MERS-CoV subgenomic mRNA vs. genomic RNA in different respiratory specimens.

2. Methods

We collected respiratory samples from 17 patients admitted to three Seoul National University (SNU) affiliated hospitals during the 2015 MERS outbreak in Korea. The patients were categorized into severe (A–I) or mild (J–Q) groups depending on their oxygen supplementation requirements. The severe group required oxygen supplementation to maintain

arterial saturation above 90 percent. Patients A-E received ventilator therapy, while patients F-I did not.⁴ The institutional review board at SNU Hospital provided study approval and waived the requirement for written consent.

Oropharyngeal and nasopharyngeal swabs were collected by using UTM™ kit containing viral transport media (Copan Diagnostics Inc., Murrieta, CA). The RNA was extracted from respiratory samples using the QIAamp viral RNA mini kit (QIAGEN, Valencia, CA). To detect the MERS-CoV genomic RNA, multiplex quantitative real-time reverse transcription (rRT)-PCR was performed using the PowerChek™ MERS (upE & ORF1a) Real-time PCR kit (Kogenebiotech, Seoul, South Korea) and all assays were performed using a ViiA™ 7 Real-time PCR system (Applied Biosystems®, Grand Island, NY). The results of genomic RNA titers were partly presented previously.⁵

The MERS-CoV subgenomic mRNA was detected using the AccuPower RT-PCR PreMix (Binder Inc. Alameda, CA). PCR primers were designed to detect subgenomic mRNA that codes for the spike (S) (433 bp) and nucleocapsid (N) (662 bp) proteins (Table 1). Forward primer was elaborated from the leader sequence and backward primers of 5' untranslated region (UTR)-S and 5'UTR-N were from gene sequences coding for protein S and N, respectively. The PCR reactions were carried out as follows: initial denaturation at 94°C for 5 min and 40 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. Subgenomic mRNA was sequenced using a DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) and the ABI BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, Grand Island, NY). If the sequences included the leader sequence and were consistent with the MERS-CoV genome by $\geq 98\%$ using Basic Local Alignment Search Tool (BLAST) software, they were confirmed as subgenomic mRNA.

3. Results and Discussion

In sputum or transtracheal aspirates, detection of MERS-CoV subgenomic mRNA was more frequent in the severe group than mild group (Fig 1A). Subgenomic mRNA was detected ≤ 28 days after the illness onset. In the severe group, the period for detecting subgenomic mRNA strongly correlated with the duration for detecting MERS-CoV genomic RNA (Pearson correlation coefficient = 0.803, $P = 0.009$). MERS-CoV genomic RNA titer was significantly higher in the specimens with subgenomic mRNA detection than in those where subgenomic mRNA was not detected ($P = 0.0007$) (Fig 2).

In oropharyngeal swab specimens, subgenomic mRNA was detected only in the severe group for up to 11 days after the illness onset (Fig 1B) and the period for detecting subgenomic mRNA was not significantly correlated with that for MERS-CoV genomic RNA (Pearson correlation coefficient = 0.335, $P = 0.378$). No subgenomic mRNA was detected in nasopharyngeal swab specimens (Fig 1C).

In the present study, replicative MERS-CoV was detected in sputum or transtracheal aspirate up to 4 weeks after symptom development in MERS-CoV-infected patients with severe pneumonia. This result was consistent with the findings in previous studies that tested MERS-CoV genomic RNA.^{1, 6, 7} On the basis of these results, infection prevention and control precautions should be thoroughly applied for at least 1 month after symptom onset if the patients with MERS-CoV infection have severe pneumonia.

The differences in the detection of replicative viruses between upper and lower respiratory tract specimens may have originated from differences in viral titers. Several studies demonstrated that the viral titer of MERS-CoV RNA in upper respiratory tract specimens was lower than in lower respiratory tract specimens.^{5, 6} In the present study, subgenomic mRNA was not detected in any of the nasopharyngeal specimens. The current guidelines recommend that isolation should continue until two consecutive upper respiratory tract specimens taken at least 24 h apart test negative by RT-PCR.⁸ However, the present study suggests that, if possible,

lower respiratory tract specimens should be used to determine the duration of isolation and that nasopharyngeal swab specimens should be avoided.

This study has a few limitations. First, differences in sensitivity, between the real time RT-PCR used for detecting genomic RNA and conventional RT-PCR for subgenomic mRNA, may affect the results. Second, RT-PCR methods for subgenomic mRNA were not validated elsewhere. Other methods, such as detecting live virus, should be performed to validate the methods used in this study.

In conclusion, replicative MERS-CoV was detected in lower respiratory tract specimens up to 4 weeks after symptom development, which was well correlated with detection of genomic RNA. In upper respiratory tract specimens, detection of subgenomic mRNA and genomic RNA did not correlate. These findings suggest that MERS-CoV does not replicate well in the upper respiratory tract.

Conflict of interest: none

Acknowledgments

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[a=1&ua=1](#) [accessed March 21, 2018].

Figure Legends

Fig 1. Detection of MERS-CoV subgenomic mRNA in sputum or transtracheal aspirates (A), throat swabs (B), and nasopharyngeal swabs (C). A pink dot denotes a positive RT-PCR result for MERS-CoV subgenomic mRNA, while a gray dot indicates a negative result. The blue lines indicate the duration from the symptom-onset to the last positive result for MERS-CoV genomic RNA. Patients A-I were in the severe group and patients J-Q were in the mild group. Among the severe group subjects, patients A-E received ventilator therapy, while patients F-I did not. The 5UTR-S and 5UTR-N denote the subgenomic mRNA of spike and nucleocapsid proteins, respectively.

Fig 2. MERS-CoV genomic RNA (*upE*) titers in sputum or transtracheal aspirates with vs. without subgenomic mRNA detection. The solid lines indicate means and standard errors of the mean. The dashed line indicates the detection limit.

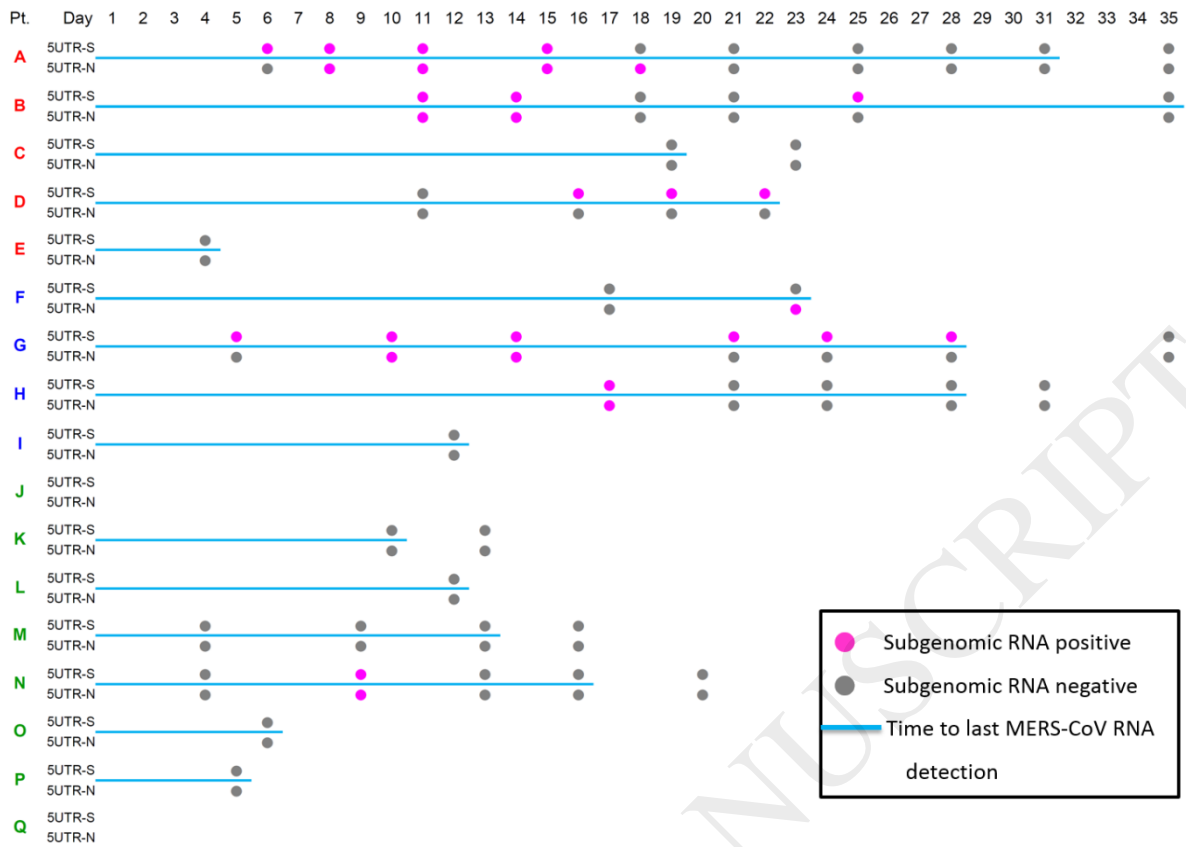


Fig 1A

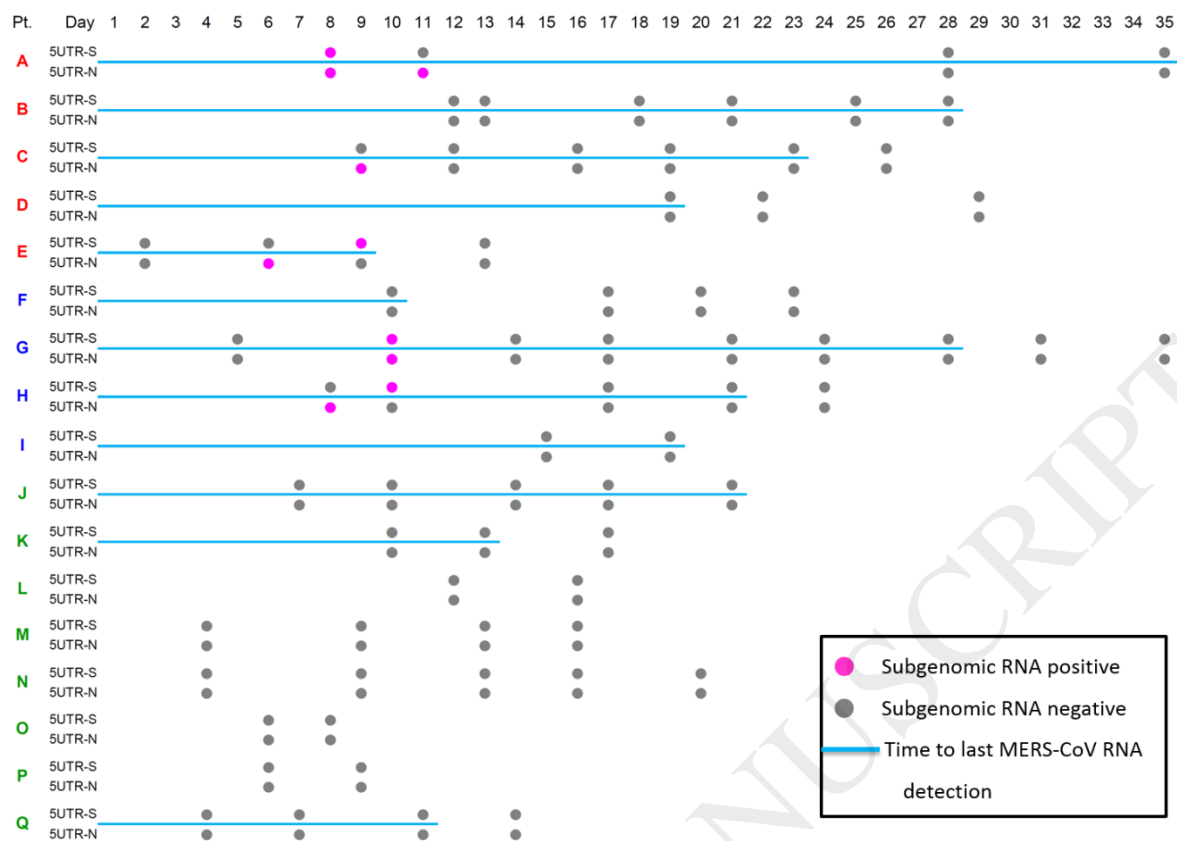


Fig 1B

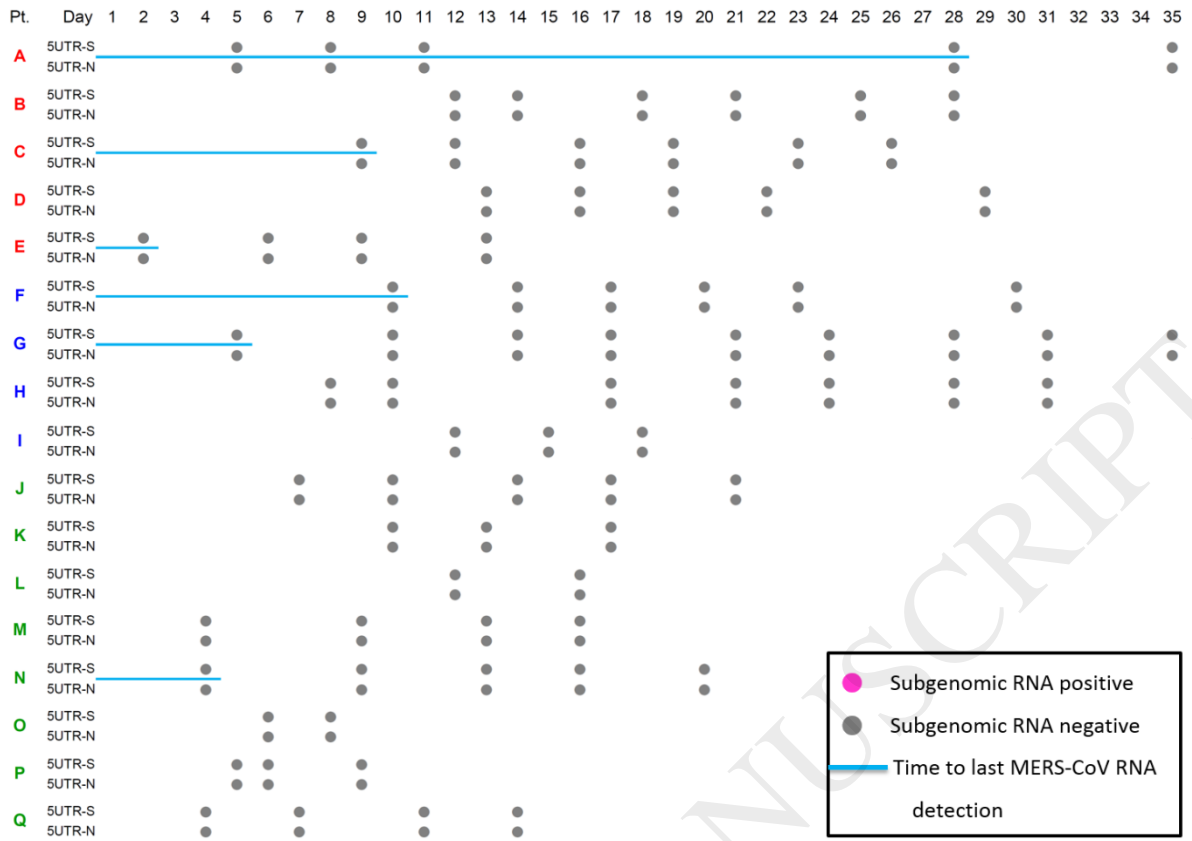


Fig 1C

Table 1. PCR primer pairs used in this study

	Forwa rd	sequence (5'→3')	Reverse	sequence (5'→3')
5'UT		GATTTAAGTGAATAGCT	S21751	TGAGAATAGTTAGCTACA
R-S	1For	TGGCTA	Rev	AACAAC
5'UT		GATTTAAGTGAATAGCT	N29121	TCCTGATCTTGAACCTTGT
R-N	1For	TGGCTA	Rev	GAAC