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Serologic responses of 42 MERS-coronavirus-infected patients according to the disease severity

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ABSTRACT

We evaluated serologic response of 42 Middle East respiratory syndrome coronavirus (MERS-CoV)-infected patients according to 4 severity groups: asymptomatic infection (Group 0), symptomatic infection without pneumonia (Group 1), pneumonia without respiratory failure (Group 2), and pneumonia progressing to respiratory failure (Group 3). None of the Group 0 patients showed seroconversion, while the seroconversion rate gradually increased with increasing disease severity (0.0%, 60.0%, 93.8%, and 100% in Group 0, 1, 2, 3, respectively; $P = 0.001$). Group 3 patients showed delayed increment of antibody titers during the fourth week, while Group 2 patients showed robust increment of antibody titer during the third week. Among patients having pneumonia, 75% of deceased patients did not show seroconversion by the third week, while 100% of the survived patients were seroconverted ($P = 0.003$).

1. Introduction

Since the first reported case of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (Zaki et al., 2012), small and large outbreaks have occurred, resulting in 1917 MERS-CoV infections and 677 related deaths to date (WHO, 2017). To understand this fatal respiratory viral infection, several serologic investigations have been conducted (Corman et al., 2016; Min et al., 2016; Park et al., 2015; Payne et al., 2016). However, practical analysis of serodiagnostic parameters for clinical usage was limited in previous studies, due to insufficient sample size or clinical information. We managed 45 MERS-CoV-infected patients, which is the largest number of patients as a single center during the 2015 Korean MERS outbreak (total 186 patients identified) (Cho et al., 2016; Kim et al., 2016; Park et al., 2016), and reported that MERS-CoV-infected patients experienced 4 distinct clinical courses, ranging from asymptomatic infection to severe pneumonia requiring mechanical ventilation (Ko et al., 2016). Based on these findings, we evaluated serologic response of 42 MERS-CoV-infected patients according to the disease severity to investigate potential role of serodiagnostic parameters as prognostic markers.

2. Material and methods

2.1. Study population and samples

Among 45 MERS-CoV-infected patients who were admitted to Samsung Medical Center, a 1950-bed tertiary care university hospital, during the 2015 Korean MERS outbreak (Ko et al., 2016), we obtained sera from 42 patients. MERS-CoV infections were confirmed on the

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basis of real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays targeting upstream of the E gene (upE) and the open-reading frame gene 1a (ORF1a) (Corman et al., 2012a; Madani, 2014). Epidemiologic investigation data and electronic medical records were reviewed to obtain exact exposure date, symptom onset, clinical course, and outcome data for the patients. One or 2 residual serum samples per week of illness were used for serologic testing during hospitalization periods. Follow-up serum samples obtained at outpatient clinics were also tested up to 6 months from symptom onset. The institutional review board of Samsung Medical Center approved the present study.

2.2. Patient grouping according to the disease severity

The clinical course of MERS-CoV-infected patients was assessed 6 weeks after symptom onset and patients were divided into 4 disease severity groups: asymptomatic infection (Group 0), symptomatic infection without pneumonia (Group 1), pneumonia without respiratory failure (Group 2), and pneumonia progressing to respiratory failure (Group 3) (Ko et al., 2016). For practical purposes, respiratory failure was defined as the need for mechanical ventilation. Only patients in Group 3 experienced fatal outcomes (5/13, 38.5%), and interval from symptom onset to death was 27 days in median (IQR 19–35.5). Proportion of underlying immunocompromising conditions including diabetes, solid cancer, or hematologic malignancies was not different between groups (Ko et al., 2016). The distinct clinical presentation of the 4 severity groups are presented in Supplementary Figs. 1 and 2, and Supplementary Table 1, in addition to the previous report (Ko et al., 2016).

2.3. Definitions

Seroconversion status was determined based on neutralization activity: if none of the serum samples from a MERS-CoV-infected patient, necessarily including sera obtained after the third week of illness, showed neutralization activity, the patient was considered to have negative seroconversion; if none of the serum samples obtained by the end of the third week of illness showed neutralization activity and no samples were available for neutralization tests thereafter, the patient was considered to have an indeterminate response (i.e. interpretation not applicable); if any serum showed neutralization activity, the patient was considered to have positive seroconversion. Patients with an indeterminate response were excluded from calculation of the seroconversion rate. This definition is based on the premise that no patients had previous exposure to MERS-CoV, as this was the first MERS outbreak in Korea as a non-endemic country.

During the outbreak, MERS-CoV exposure dates and symptom onsets were clearly identified in most patients, owing to thorough contact investigation and monitoring of exposed individuals (Cho et al., 2016; Park et al., 2016). MERS-related symptoms included fever, myalgia, cough, sputum, and diarrhea. To provide a common point of reference, we used 'days post onset of illness (*dpoi*)' to evaluate MERS-CoV-infected patients. For asymptomatic patients, the day of diagnosis of MERS-CoV infection was considered as day of symptom onset (Ko et al., 2016).

2.4. Serologic tests for MERS-CoV antibody

2.4.1. Enzyme-linked immunosorbent assay (ELISA) IgG and IgA

Anti-MERS-CoV ELISA IgG and IgA (Euroimmun, Lübeck, Germany) were based on soluble MERS-CoV spike protein S1 domain expressed in HEK-293 T cells (Muller et al., 2014, 2015; Muth et al., 2015; Raj et al., 2013). Sera were tested according to the manufacturer's instructions with 1:100 dilutions. Secondary detection was done with peroxidase-labeled anti-human IgG and IgA. Cutoff values of OD ratio 0.4 for ELISA IgG and 0.2 for ELISA IgA were applied in the present study, as these values exhibited optimal performance in predicting neutralization activity (Ko et al., 2017).

2.4.2. IFA IgM

Anti-MERS-CoV IFA IgM (Euroimmun) was performed with slides carrying Vero cells infected with full MERS-CoV (Corman et al., 2012b; Meyer et al., 2014; Muller et al., 2014, 2015). Sera were tested according to the manufacturer's instructions with 1:10 dilutions. Weekly positive IFA intensity was considered cutoff intensity value of IFA IgM, which exhibited optimal performance in predicting neutralization activity (Ko et al., 2017).

2.4.3. PRNT

MERS-CoV PRNT was performed as previously described (Meyer et al., 2014; Muller et al., 2014, 2015). Pre-dilution before setting up the log2-dilution series was 1:10, defining 1:20 as the lowest possible significant titer for categorizing a sample as positive (Meyer et al., 2014).

2.5. Statistical analysis

For comparison of clinical variables between groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis test was used for continuous variables, and chi-square or Fisher's exact test was used for categorical variables. Six-week survival probability was calculated using the Kaplan–Meier method. The Cox proportional hazard model and log-rank test were used to examine the association of seroconversion status with the 6-week mortality of MERS patients having pneumonia. All *P*-values were 2-tailed, and those <0.05 were considered to be statistically significant. R-3.3.1 for Windows (RStudio, Boston, MA, USA) was used for all statistical analyses.

3. Results

3.1. Serologic response of MERS-CoV infection according to the disease severity

Seroconversion status of 42 MERS-CoV-infected patients is summarized in Table 1. None of the Group 0 patients showed seroconversion, and the seroconversion rate gradually increased with increasing disease severity (0.0%, 60.0%, 93.8%, and 100% in groups 0, 1, 2, and 3, respectively; $P = 0.001$). Seroconversion was observed from 14 to 24 *dpoi* (18 *dpoi* in median), mostly during the third week of illness (88.0% of seroconverted patients with a known timeline). Group 3 patients showed slightly delayed timing of seroconversion compared to Group 2 patients (18.5 and 17.5 *dpoi* in median, respectively, without statistical significance), and seroconversion during the fourth week of illness was exclusively observed in Group 3.

Serologic responses of seroconverted patients are depicted according to the severity groups with 7-day intervals in Fig. 1. Serologic response occurred from the third week of illness, and antibody response is weaker in patients with mild symptomatic patients (Group 1) than patients with pneumonia (Groups 2 and 3). Group 2 patients showed robust increment of antibody titer during the third week (compared to the 2nd week, the median OD ratios of ELISA IgG and IgA increased more than 3-fold, and IFA IgM and PRNT increased from negative to 2+ and 1:80, respectively), and the titers did not significantly increase thereafter (in comparison of the median values of third week and fourth week, no statistical significance was observed). Meanwhile, Group 3 patients showed delayed and continuous increment of antibody titers from the third week: the median values of each serologic test were significantly higher during the fourth week compared to those of the third week in Group 3 (all $P < 0.05$). In comparison between Groups 2 and 3, antibody titers of Group 3 patients during the third week were numerically lower than those of Group 2, although only ELISA IgG showed statistically significant difference ($P = 0.016$). The antibody titers of Group 3 patients continuously increased, showing numerically higher titers

Table 1
Seroconversion status of MERS-CoV-infected patients according to the disease severity group.

Variables	Classification by the disease severity			
	Group 0 *Asymptomatic* (n = 3)	Group 1 *Symptomatic* (n = 10)	Group 2 *Pneumonia* (n = 18)	Group 3 *Resp. failure* (n = 11)
Negative seroconversion	3 (100%)	2 (20.0%)	1 (5.6%)	0 (0.0%)
Indeterminate response	0 (0.0%)	5 (50.0%)	2 (11.1%)	2 (18.2%)
Positive seroconversion	0 (0.0%)	3 (30.0%)	15 (83.3%)	9 (81.8%)
Timing unknown*	N/A	0/3 (0.0%)	1/15 (6.7%)	1/9 (11.1%)
Second week of illness	N/A	0/3 (0.0%)	1/14 (7.1%)†	0/8 (0.0%)
Third week of illness	N/A	3/3 (100%)	13/14 (92.9%)	6/8 (75.0%)
Fourth week of illness	N/A	0/3 (0.0%)	0/14 (0.0%)	2/8 (25.0%)
dpoi	N/A	17 (16–18)	17.5 (14–20)	18.5 (15–24)
dpex	N/A	22 (20–24)	21.5 (19–30)	24 (18–27)
Seroconversion rate‡	0/0 (0.0%)	3/5 (60.0%)	15/16 (93.8%)	9/9 (100%)

Data are expressed as the number (%) of patients or median (range).
Seroconversion was confirmed by PRNT and a 1:20 dilution was defined as the lowest significant titer.
Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; Resp., respiratory; *dpoi*, days post onset of illness; *dpex*, days post exposure; IQR, interquartile range; PRNT, plaque reduction neutralization test.
* The timing of seroconversion was uncertain for 2 patients as the only sera available were collected after several months (*dpoi* 79 and 140).
† At *dpoi* 14.
‡ Patients with an indeterminate response were excluded from seroconversion rate analysis.

compared to those of Group 2 patients during the fourth week (without statistical significance). Detailed serologic test results for each patient are presented according to timeline and severity groups in Supplementary Tables 2 to 5.

3.2. Impaired Serologic Response of Fatal MERS Pneumonia

As seroconversion rates were low in mild severity groups (0% in Group 0 and 60% in Group 1), outcome analysis was performed in patients having pneumonia (Groups 2 and 3). Only 25% of deceased patients showed seroconversion by the end of the third week of illness, while 100% of survived patients seroconverted ($P = 0.003$, Table 2). This difference also could be discriminated by ELISA IgG (with OD ratio cutoff value of 0.4, $P = 0.003$) and ELISA IgA (with OD ratio cutoff value of 0.2, $P = 0.010$). IFA IgM response was not significantly different between survivors and non-survivors (with intensity cutoff value of weakly positive, $P = 0.135$). In a Kaplan–Meier analysis comparing seroconverted patients and non-converted patients by the third week of illness, seroconverted patients showed significantly higher survival probability compared to patients with negative seroconversion (Fig. 2, $P < 0.001$ by log-rank test). Negative seroconversion in pneumonia patients by the third week of illness showed a hazard ratio of 27.83 (95% CI 2.76–280.21, $P = 0.005$, by the Cox proportional hazard model) in predicting 6-week mortality.

4. Discussion

Since previous hospital-associated outbreaks of MERS occurred in endemic countries, where primary infections flow from community into hospitals, detailed clinical data of each patient were hard to obtain (Corman et al., 2016). However, during the 2015 Korean MERS outbreak, the first outbreak in a non-endemic country, epidemiologic links and entire clinical course of each patients could be clearly identified (Ko et al., 2016; Park et al., 2016). Owing to the detailed epidemiologic and clinical information about patients, we could find out different serologic response depending on disease severity and outcome.

Although different seroconversion rates depending on disease severity can be inferred from previous serologic investigation (Min et al., 2016), the number of evaluated MERS patients was limited to 14 and neutralization testing was not performed. In that study, a robust increment of ELISA IgG titer with a 3-fold increase in OD ratio was exclusively observed among patients with severe pneumonia, while mild infections exhibited a modest increment in OD ratio, if any. Likewise, we noted that asymptomatic MERS-CoV-infected cases did not show serologic response including PRNT within 6 months, and the seroconversion rate increased with the disease severity. Although the number of asymptomatic patients was limited to 3 in the present analysis, it is less likely that asymptomatic patients will experience seroconversion considering that even Group 1 patients with obvious MERS-related symptoms showed low seroconversion rate of 60%. This finding correlates with another serologic study that evaluated 11 rRT-PCR-confirmed MERS patients (Choe et al., 2017). In that study, antibody titers in 4 of 6 patients with mild illness were undetectable. In addition, most contact surveys of MERS-CoV could not detect additional rRT-PCR-negative PRNT-positive MERS-CoV infections (Breakwell et al., 2015; Buchholz et al., 2013; Choi et al., 2016; Ko et al., 2017). These findings imply that serologic surveys to detect subclinical infections among asymptomatic individuals would not be effective.

Serologic response was delayed in Group 3 patients, and negative seroconversion by the third week of illness was associated with fatal outcome among patients with MERS pneumonia (HR 27.83, 95% CI 2.76–280.21, $P = 0.005$). Delayed commencement of serologic response in severe disease was also suggested by previous report by Park et al. (Park et al., 2015). Although seroconversion timing was not statistically significantly delayed in Group 3 patients in the present study, delayed increment of IgG, IgA, and IgM titers after the third week was demonstrated in Group 3. However, the delayed serologic response in Group 3 could not be used as predictor for respiratory failure, as respiratory failure progressed during the 2nd week of illness (12 *dpoi* in median).

Meanwhile, negative seroconversion in MERS pneumonia by the third week of illness was associated with fatal outcome in the present analysis. Impaired serologic response in deceased patient was also noted in the paper of Corman et al., but insufficient clinical information, especially day of symptom onset, hampered more detailed analysis in association with timeline (Corman et al., 2016). In this study, we could obtain exact clinical information including day of symptom onset, and figured it out that seroconversion status by the third week of illness (by 21 *dpoi*) can serve as a prognostic marker. Another important point is that MERS-CoV-infected patients in the present analysis died later than previous reports, probably owing to antiviral therapy or aggressive critical care including extracorporeal membrane oxygenation (ECMO). The median interval from symptom onset to death was 27 days in the present study, which is much longer than 11.5 days in previous reports (Zumla et al., 2015). Although rapidly deteriorating MERS cases would die before the third week of illness, there certainly is a population that benefit from prognosis prediction by serologic response. Aggressive managements including ECMO should be considered for pneumonia patients without seroconversion by the third week of illness. Although seroconversion status can be confirmed by neutralization tests, it cannot be readily performed worldwide (Corman et al.,

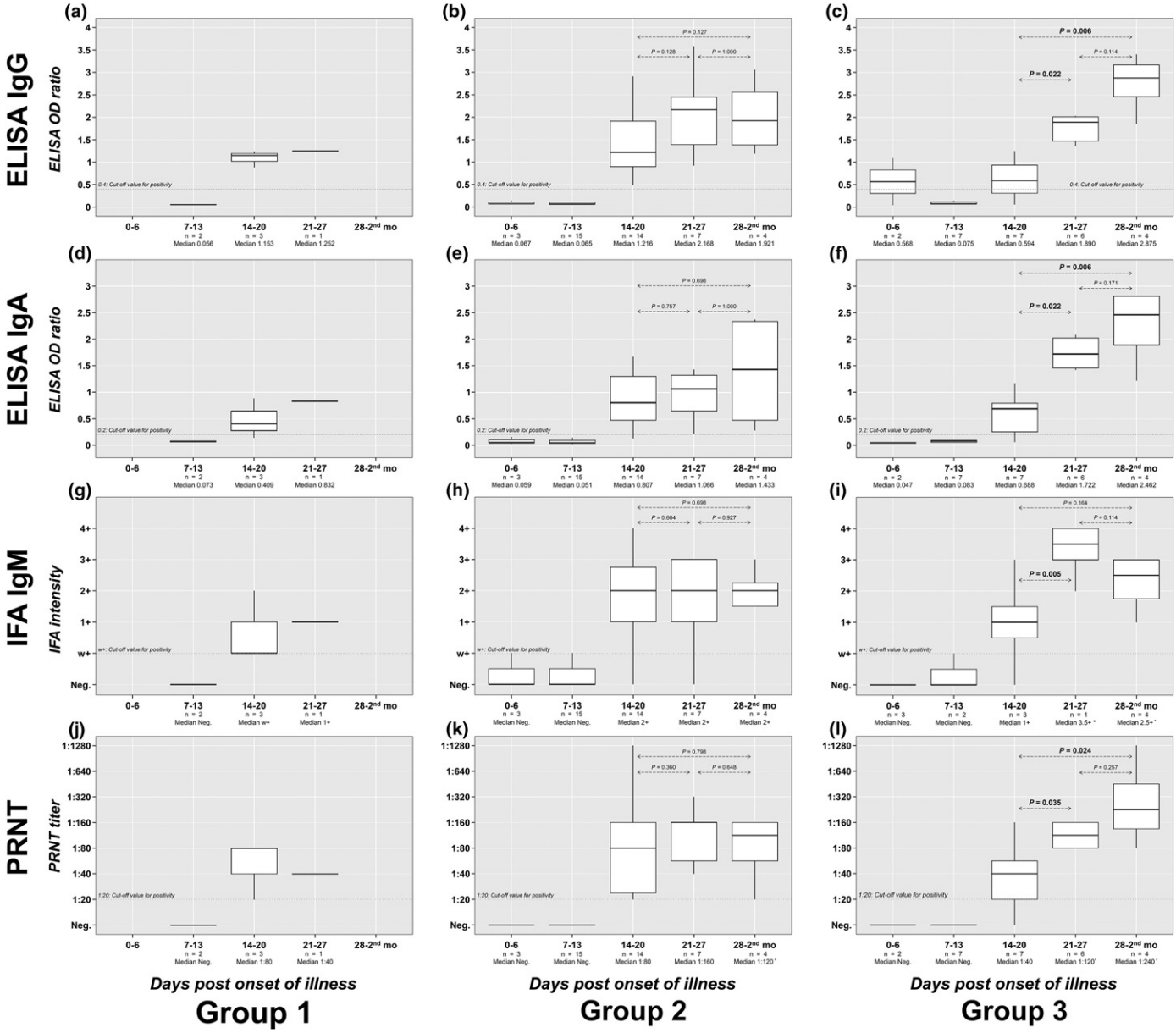

Fig. 1. Serologic responses of seroconverted MERS-CoV-infected patients, according to the severity groups with 7-day intervals. The serologic responses of seroconverted MERS-CoV-infected patients are depicted according to the severity groups: symptomatic infection without pneumonia (Group 1), pneumonia without respiratory failure (Group 2), and pneumonia progressing to respiratory failure (Group 3). The mean values of each serologic test for 7-day intervals are presented in box-plots. The antibody titers of symptomatic patients rise after the 2nd week. Although PRNT titers were not statistically different between groups by the third week of illness, peak antibody response increased as severity increases. (a) ELISA IgG in Group 1. (b) ELISA IgG in Group 2. (c) ELISA IgG in Group 3. (d) ELISA IgA in Group 1. (e) ELISA IgA in Group 2. (f) ELISA IgA in Group 3. (g) IFA IgM in Group 1. (h) IFA IgM in Group 2. (i) IFA IgM in Group 3. (j) PRNT in Group 1. (k) PRNT in Group 2. (l) PRNT in Group 3. Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; ELISA, enzyme linked immunosorbent assay; OD, optical density; IFA, immunofluorescence assay; PRNT, plaque reduction neutralization test.

Table 2
Seroconversion rates by the end of third week of illness according to outcome of MERS-CoV-infected patients having pneumonia (Groups 2 and 3).

Serologic tests	Survived (n = 18)	Deceased (n = 4)	*P* value
PRNT (≥ 1:20 dilution)	18 (100%)	1 (25%)	0.003
ELISA IgG (OD ratio cutoff ≥0.4)	18 (100%)	1 (25%)	0.003
ELISA IgA(OD ratio cutoff ≥0.2)	17 (94.4%)	1 (25%)	0.010
IFA IgM (Intensity cutoff ≥ w+)	16 (88.9%)	2 (50%)	0.135

Data are expressed as the number (%) of patients. The population of this analysis is 22 MERS-CoV-infected patients with pneumonia (Groups 2 and 3) whose sera were collected during the third week of illness.
Abbreviation: MERS-CoV, Middle East respiratory syndrome coronavirus; PRNT, plaque reduction neutralization test; ELISA, enzyme-linked immunosorbent assay; OD, optical density; IFA, immunofluorescence assay; w+, weak positive.

2016). In the present analysis, seroconversion status of the third week assessed by ELISA IgG and IgA was similar with that by PRNT. These ELISA tests can be practically used for predicting poor prognosis of MERS pneumonia in the field of patient management.

As a retrospective study, serum samples of each patient could not be collected with same interval. However, we applied strict criteria for seroconversion, excluding patients who did not have follow-up samples after the third week of illness as indeterminate response. In the previous report with the same patient population, we also suggest predictive factors for disease progression using clinical variables within 3 days from symptom onset (Ko et al., 2016). Together with the present paper, these factors could be used complementarily in managing MERS-CoV-infected patients. In addition, although we identified that seroconversion status by

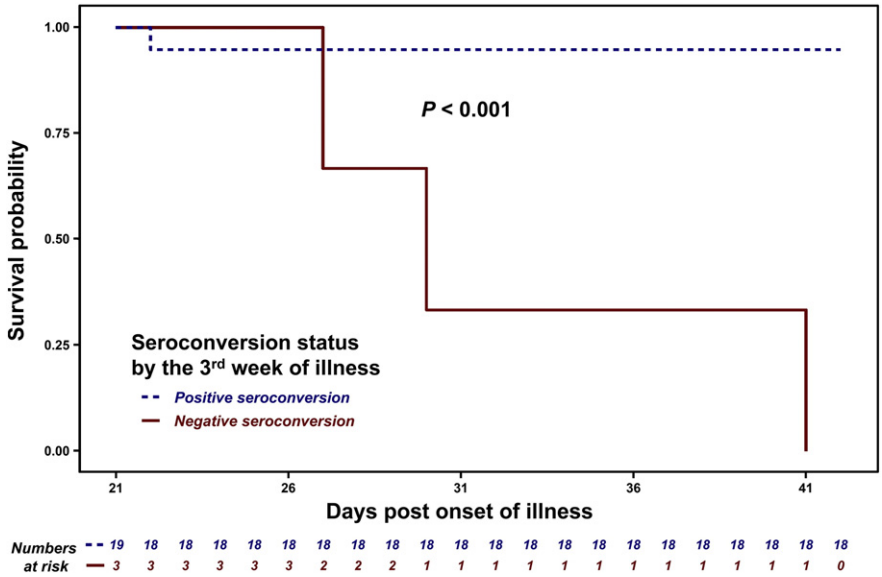

Fig. 2. Survival probability of MERS-CoV-infected patients having pneumonia according to the seroconversion status by the third week of illness. Survival probability according to the seroconversion status was evaluated in MERS-CoV-infected patients having pneumonia, whose seroconversion status during the third week of illness is identifiable. Seroconverted patients showed significantly higher survival probability compared to patients with negative seroconversion ($P < 0.001$ by log-rank test). Seroconversion was confirmed by PRNT and a 1:20 dilution was defined as the lowest significant titer. Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; PRNT, plaque reduction neutralization test.

the third week was associated with fatal outcomes of MERS pneumonia, we could not perform multivariate analysis due to limited sample size. This finding need to be further evaluated with enough patient numbers of MERS pneumonia.

5. Conclusions

In conclusion, in a serologic investigation of 42 MERS-CoV-infected patients, mild cases showed low seroconversion rates, while fatal cases showed impaired serologic responses.

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Conflicts of Interest

There are no potential conflicts of interest relevant to this article to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.diagmicrobio.2017.07.006.

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