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Short communication

Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience

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ABSTRACT

Background: The effects of convalescent plasma (CP) infusion, one of treatment options for severe Middle East respiratory syndrome coronavirus (MERS-CoV) infections, have not yet been evaluated.

Methods: Serologic responses of CP-infused MERS patients during the 2015 Korean MERS outbreak at a tertiary care center were evaluated. Serologic activity was evaluated with anti-MERS-CoV enzyme-linked immunosorbent assay (ELISA) IgG, ELISA IgA, immunofluorescence assay IgM, and plaque reduction neutralization test (PRNT). Donor plasma and one or two recipient's serum samples per week of illness including one taken the day after each CP infusion were evaluated. For sensitivity and specificity analysis of ELISA IgG in predicting neutralization activity, a data set of 138 previously evaluated MERS-CoV-infected patients was used.

Results: Three of 13 MERS patients with respiratory failure received four CP infusions from convalesced MERS-CoV-infected patients, and only two of them showed neutralizing activity. Donor plasma with a PRNT titer 1:80 demonstrated meaningful serologic response after CP infusion, while that with PRNT titer a 1:40 did not. ELISA IgG predicted neutralization activity of a PRNT

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titer ≥ 1:80 with more than 95% specificity at a cut-off optical density (OD) ratio of 1.6, and with 100% specificity at an OD ratio of 1.9.

Conclusions: For effective CP infusion in MERS, donor plasma with a neutralization activity of a PRNT titer ≥ 1:80 should be used. ELISA IgG could substitute for the neutralization test in resource-limited situations.

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Running head: Convalescent plasma infusion in MERS

INTRODUCTION

Convalescent plasma (CP) infusion is one treatment option for severe Middle East respiratory syndrome coronavirus (MERS-CoV) infections, but the effect of CP infusion in MERS has not yet been evaluated [1]. During the 2015 Korean MERS outbreak, we performed CP infusions in three patients with severe MERS-CoV infection and evaluated serologic responses of the CP recipients after the end of the outbreak.

METHOD

Study population and CP transfusion

During the 2015 Korean MERS outbreak, 45 MERS-CoV infected patients were admitted to a 1,950-bed tertiary care university hospital [2]. Thirteen patients progressed to respiratory failure, and 3 of them (23.1%), the most clinically severe patients at the time the CP was prepared, received CP infusions [3].

CP donors were MERS-CoV-infected patients during the Korean outbreak, whose recovery was confirmed by two consecutively negative sputum MERS-CoV polymerase chain reaction (PCR) assays and resolution of clinical symptoms. The donor's blood was collected within their 3rd week of illness and screened for, MERS-CoV PCR, and serology for hepatitis B and C virus, human immunodeficiency virus, and syphilis. One or two serum samples per week of illness, including one taken the day after CP infusion were used for serologic evaluation of CP recipients. For performance analysis of enzyme-linked immunosorbent assay (ELISA) IgG in predicting neutralization activity, a data set of 138 previously evaluated MERS-CoV-infected patients was used [4]. The Institutional Review Board of Samsung Medical Center approved the present study.

Serologic tests for MERS-CoV antibody

Anti-MERS-CoV ELISA IgG, ELISA IgA, and immunofluorescence assay (IFA) IgM (Euroimmun, Lübeck, Germany) were performed as previously described [5–7] with cut-off values of optical density (OD) ratio 0.4 for ELISA IgG and 0.2 for ELISA IgA [4]. To assess neutralization activity, a MERS-CoV plaque reduction neutralization test (PRNT) was performed as previously described [6].

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RESULT

Serologic response of MERS patients treated with CP infusion

Patient A (Figure 1-a)

A previously healthy 55-year-old male experienced was diagnosed with MERS-CoV infection, and developed pneumonia on 8 days post onset of illness (*dpoi*). He received mechanical ventilation support on *dpoi* 10 and CP infusion #1 on *dpoi* 11. Serologic tests of donor plasma #1 were positive for IFA IgM (weakly positive) and PRNT (titer 1:40), while negative for ELISA IgG (OD ratio 0.338) and ELISA IgA (OD ratio 0.079). Follow-up recipient serum samples taken one and three days after CP infusion #1 did not show seroconversion. On *dpoi* 18, he received CP infusion #2 from a different donor. Although a follow-up recipient serum sample taken the day after CP infusion #2 showed a positive serologic response to ELISA IgG (OD ratio 2.132) and PRNT (titer 1:160), donor plasma #2 was negative to serologic tests except IFA IgM. Patient A recovered from MERS and was discharged on *dpoi* 33.

Patient B (Figure 1-b)

A previously healthy 32-year-old male was diagnosed with MERS-CoV infection, and developed pneumonia on *dpoi* 4. He received endotracheal intubation for mechanical ventilation on *dpoi* 6, and received a CP infusion on *dpoi* 8. Two hours after the CP infusion, his oxygenation suddenly worsened with aggravated radiologic infiltrations. Since MERS-CoV can also result in acute lung injury, he was diagnosed as possible transfusion-related acute lung injury (TRALI) [8]. Donor plasma was negative in all serologic tests for MERS-CoV, and a follow-up recipient serum sample taken the day after the CP infusion did not show any serologic response. From *dpoi* 13, the ELISA IgG OD ratio began to increase, and the patient's serum showed neutralizing activity on *dpoi* 16. He recovered from MERS without specific management for TRALI such as corticosteroids, and was discharged on *dpoi* 102.

Patient C (Figure 1-c)

A previously healthy 32-year-old male was diagnosed with MERS-CoV infection. He developed pneumonia on *dpoi* 5, and was intubated on *dpoi* 7. Although his early serologic tests were positive for ELISA IgG (OD ratio 1.091 on *dpoi* 2, and 0.621 on *dpoi* 9), they were considered false-positive reactions because other serologic tests except ELISA IgG were negative, ELISA IgG OD ratio decreased in follow-up samples, and he did not have a previous history of exposure to MERS-CoV nor a travel history to MERS endemic countries. On *dpoi* 14, his oxygenation worsened, and he received extra-corporeal membrane oxygenator (ECMO) support and a CP infusion. The donor plasma was positive for ELSIA IgG (OD ratio 1.243), IgA (OD ratio 0.409), IFA IgM (one positive), and PRNT (titer 1:80). The follow-up recipient serum taken the day after the CP infusion was sero-positive (PRNT titer 1:20) on *dpoi* 15. ECMO was removed on *dpoi* 21 and he was discharged on *dpoi* 39.

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Cut-off value of ELISA IgG in predicting PRNT titer ≥ 1:80

Since only Patient C was presumed to experience a meaningful serologic response after infusion of CP with neutralization activity of PRNT titer 1:80, the performance of ELISA IgG for prediction of PRNT titer ≥ 1:80 was evaluated using 138 serum samples of MERS-CoV-infected patients (Table 1). From the cut-off value of OD ratio 1.6, ELISA IgG could predict PRNT titer ≥ 1:80 with a specificity over 95%. 100% specificity was noted at a cut-off value of OD ratio 1.9.

DISCUSSION

Passive immunization including CP infusion is a potential therapeutic option in emerging viral infections without efficacy-proven antiviral agents [9]. Although retrospective studies of severe acute respiratory infections (SARI) of viral etiology have yielded controversial results, a meta-analysis found a significant reduction of mortality with CP infusions [10]. Based on these reports, CP infusion was suggested for the treatment of MERS-CoV infection [1], and was actually tried in several cases during the 2015 Korean outbreak. Our experience suggests several challenging points of CP infusion in MRES-CoV.

First, only two of four donor plasmas (50%) used in CP infusions showed neutralizing activity. Since we used the plasma of convalesced patients who recovered from MERS-CoV infection during the early phase of the 2015 Korean outbreak, all donors experienced mild disease. MERS-CoV infection showed low seroconversion rates, especially with mild diseases [11]. MERS-CoV-infected patients without pneumonia development showed 60% seroconversion rate, while 96% of pneumonic patients showed seroconversion [12]. This difference suggests that donors should be tested for antibody titers, otherwise be selected from patients who recovered from severe illness.

Second, only Patient C, who received donor plasma with a PRNT titer of 1:80, demonstrated a meaningful antibody response after CP infusion; he showed seroconversion the day after the CP infusion on *dpoi* 15, which is the earliest reported response among MERS-CoV infected patients who progressed to respiratory failure [12]. However, Patient A did not exhibit seroconversion after CP infusion with a PRNT titer of 1:40, and seroconversion occurred on *dpoi* 19, the median timing [12]. Although the seroconversion in Patient C might not be related with CP and our experience is limited in a few cases, a possibility could be suggested that donor plasma needs neutralizing activity of a minimum PRNT titer of 1:80 to achieve a meaningful serologic response after CP infusion. Previous efficacy-proven studies of CP infusion have selectively used CP with high neutralizing activity and emphasized dose of neutralizing antibodies [13–15]. However, since neutralization test for MERS-CoV require facilities of biosafety level 3, the ability to measure neutralization activity may be limited during an unexpected outbreak. In contrast, ELISA IgG can be relatively easily performed. Accordingly, we also evaluated the performance of ELISA IgG that was associated with a neutralization activity with PRNT titer ≥ 1:80. With an ELISA IgG cut-off value of OD ratio 1.6, ELISA

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IgG could predict PRNT titer ≥ 1:80 with more than 95% specificity, and with and OD ratio of 1.9, prediction occurred with 100% specificity.

Third, our cases received an initial CP infusion after progression to respiratory failure, on *dpoi* 8 to 14. Because MERS-CoV infection progresses stepwise [3], CP infusions would be inevitably delayed if only patients with progressed disease were selected as recipients. Since previous studies of SARI suggest that early infusion is more beneficial [10], potentially severe patients should be selected for CP infusion before progression to respiratory failure. During the 2015 MERS outbreak, we observed that initial clinical presentation, including age, high viral load, body temperature, and laboratory findings, could predict disease progression [3]. Although such variables could be modified depending on clinical setting, we think recipient selection criteria based on initial presentation should be established to provide early CP infusion to potentially severe MERS patients.

Although all three patients with CP infusion survived, it is limited to evaluate the treatment's effectiveness, since only one infusion probably provoked a meaningful serologic response. Other CP infusion performed in others centers during the outbreak also did not show meaningful results [16,17]. Therefore, the efficacy of CP infusion in MERS-CoV infection should be evaluated in MERS-CoV endemic countries with a well-designed protocol [18]. Our experience can help CP infusion protocol establishment, in addition to management of other potential MERS outbreaks in the future.

In conclusion, for effective CP infusion in MERS-CoV infection, donor plasma should be tested for antibody activity and neutralization activity of a PRNT titer ≥ 1:80 might be required. ELISA IgG could substitute for neutralization tests in resource-limited situations.

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Disclosure statement

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

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FIGURE LEGEND

Figure 1. Serologic response of three MERS patients who received CP infusion

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Viral loads are presented as 1/Ct value. 1/Ct value of 1/40 was lower detection limit of the rRT-PCR. WBC is presented as cells/mm^3, platelet count as 10^{3}cells/mm^3, and CRP as mg/dL.

(a) A previously healthy 55-year-old male patient who progressed to respiratory failure after MERS-CoV infection. He did not show a serologic response after CP infusion #1, and CP #2 did not contain neutralization activity.

(b) A previously healthy 32-year-old male patient who progressed to respiratory failure after MERS-CoV infection. Donor plasma did not contain neutralization activity, and he experienced possible TRALI.

(c) A previously healthy 32-year-old male patient who progressed to respiratory failure and received ECMO support after MERS-CoV infection. The donor plasma had neutralization activity with PRNT titer 1:80, and the patient showed seroconversion the day after the CP infusion.

Abbreviations: MERS, Middle East respiratory syndrome; CP, convalescent plasma; Ct, threshold cycle; rRT-PCR, real-time reverse transcriptase polymerase chain reaction; WBC, white blood cell; CRP, C-reactive protein; TRALI, transfusion-related acute lung injury; ECMO, extracorporeal membrane oxygenation; PRNT, plaque reduction neutralization test

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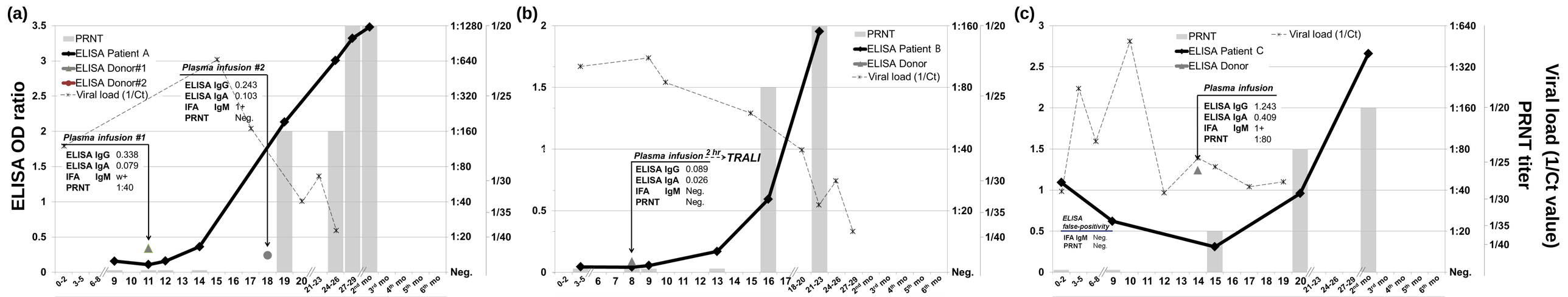
Table 1. Performance of anti-MERS-CoV ELISA IgG in predicting PRNT titer ≥ 1:80 in 138 MERS-CoV-infected patients

Predictive values	**Cut-off OD ratio for PRNT ≥ 1:80**			
	≥ 0.4	**≥ 0.9**	**≥ 1.6**	**≥ 1.9**
Sensitivity	100%	92.7%	70.7%	61.0%
Specificity	73.2%	84.5%	96.9%	100%
PPV	61.2%	71.7%	90.6%	100%
NPV	100%	96.5%	88.7%	85.8%
Sensitivity + specificity	173.2	177.2	167.6	161.0

Data are expressed as a percentage of each predictive value according to various OD ratio cut-offs. AUC calculated by the ROC curve was 0.958.

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; ELISA, enzyme-linked immunosorbent assay; OD, optical density; PRNT, plaque reduction neutralization test; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; ROC, receiver operating characteristic



Clinical Course	Fever / Pneumonia		MV			MV weaning		Discharge
WBC	4370	2690	2350	8590	2940	5770	3970	4310
Platelet	192	119	104	102	97	134	239	412
CRP	0.33	0.75	2.43	7.52	22.94	23.59	8.16	2.55

Clinical Course	Fever / Pneumonia		MV	TRALI			MV weaning	Discharge
WBC	6490	4180	4930	11400	2980	4790	4040	
Platelet	180	135	124	126	124	198	355	
CRP	8.05	7.23	8.87	11.87	7.12	7.20	0.71	

Clinical Course	Fever / Pneumonia		MV	ECMO insertion		ECMO removal	Discharge
WBC	5210	2160	2320	8400	11400	10800	
Platelet	177	94	126	89	125	210	
CRP	1.33	10.35	5.12	23.87	15.29	13.27	