Short communication

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

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Background: The effects of convalescent plasma (CP) infusion, one of the treatment options for severe Middle East respiratory syndrome coronavirus (MERS-CoV) infections, have not yet been evaluated.
Methods: Serological responses of CP-infused MERS patients during the 2015 Korean MERS outbreak at a tertiary care centre were evaluated. Serological activity was evaluated with anti-MERS-CoV enzyme-linked immunosorbent assay (ELISA) immunoglobulin (Ig)G, 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 thirteen 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 titre 1:80 demonstrated meaningful serological response after CP infusion, while that with a PRNT titre 1:40 did not. ELISA IgG predicted neutralization activity of a PRNT titre ≥1:80 with more than 95% specificity at a cutoff 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 titre ≥1:80 should be used. ELISA IgG could substitute for the neutralization test in resource-limited situations.

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 serological responses of the CP recipients after the end of the outbreak.

Methods

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 three 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 HBV, HCV, HIV and syphilis. One or two serum samples per week of illness, including one taken the day after CP infusion were used for serological evaluation of CP recipients. For performance analysis of enzyme-linked immunosorbent assay (ELISA) immunoglobulin (Ig)G 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.

Serological 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 cutoff 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].

Results

Serological response of MERS patients treated with CP infusion

Patient A

A previously healthy 55-year-old male was diagnosed with MERS-CoV infection and developed pneumonia at 8 days post onset of illness (dpoi; Figure 1A). He received mechanical ventilation support on dpoi 10 and CP infusion #1 on dpoi 11. Serological tests of donor plasma #1 were positive for IFA IgM (weakly positive) and PRNT (titre 1:40), while negative for ELISA IgG (OD ratio 0.338) and ELISA IgA (OD ratio 0.079). Follow-up recipient serum samples taken 1 and 3 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 serological response to ELISA IgG (OD ratio 2.132) and PRNT (titre 1:160), donor plasma #2 was negative to serological tests except IFA IgM. Patient A recovered from MERS and was discharged on dpoi 33.

Patient B

A previously healthy 32-year-old male was diagnosed with MERS-CoV infection and developed pneumonia on dpoi 4 (Figure 1B). He received endotracheal intubation for mechanical ventilation on dpoi 6 and received a CP infusion on dpoi 8. 2 h after the CP infusion, his oxygenation suddenly worsened with aggravated radiological 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 serological tests for MERS-CoV and a follow-up recipient serum sample taken the day after the CP infusion did not show any serological 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.

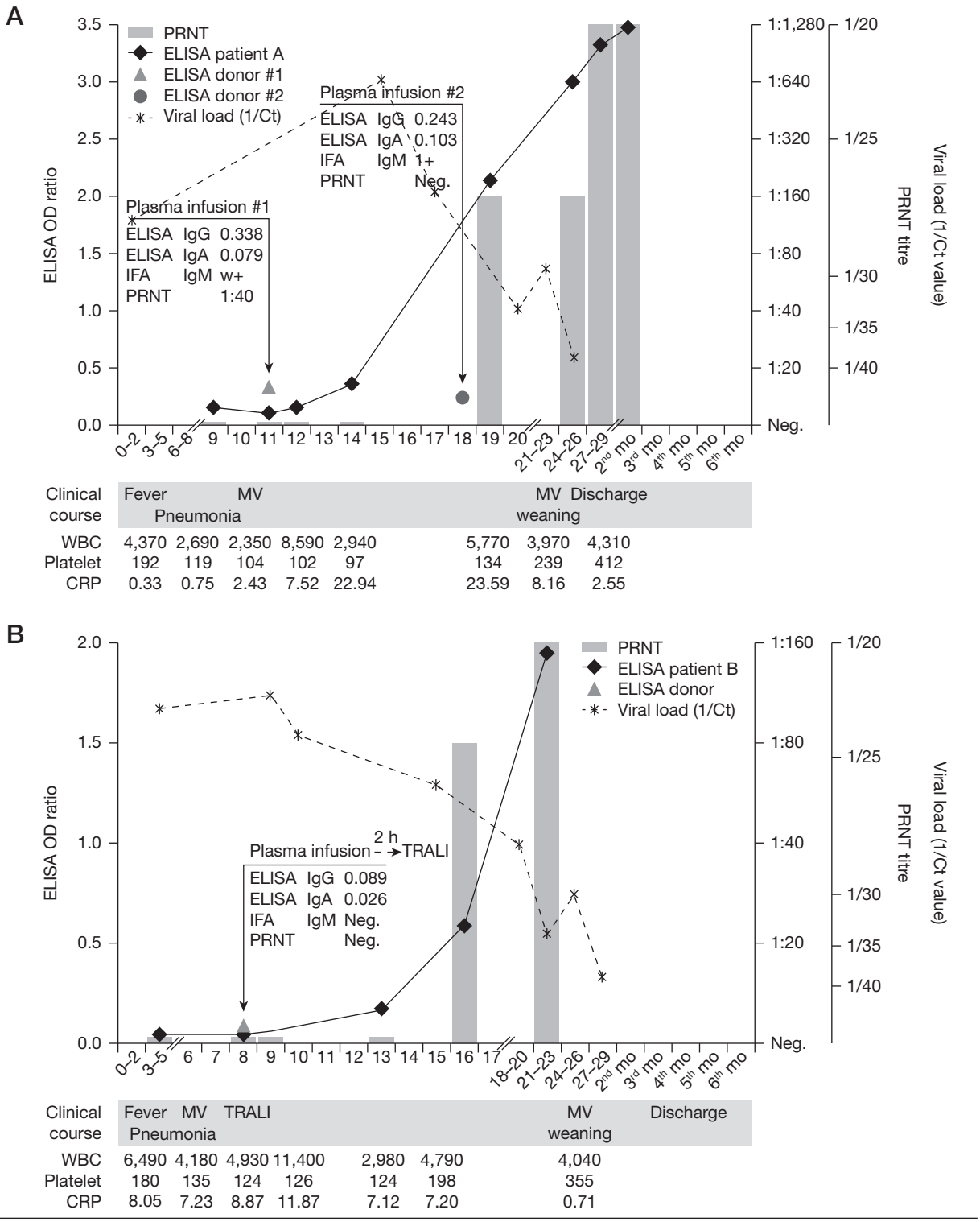
Patient C

A previously healthy 32-year-old male was diagnosed with MERS-CoV infection (Figure 1C). He developed pneumonia on dpoi 5 and was intubated on dpoi 7. Although his early serological 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 serological 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 (titre 1:80). The follow-up recipient serum taken the day after the CP infusion was sero-positive (PRNT titre 1:20) on dpoi 15. ECMO was removed on dpoi 21 and he was discharged on dpoi 39.

Cutoff value of ELISA IgG in predicting PRNT titre ≥1:80

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

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



Viral loads are presented as 1/threshold cycle (Ct) value. 1/Ct value of 1/40 was lower detection limit of the real-time reverse transcriptase polymerase chain reaction (rRT-PCR). White blood cells (WBC) are presented as cells/mm^3, platelet count as 10^3 cells/mm^3 and C-reactive protein (CRP) as mg/dl. **(A)** A previously healthy 55-year-old male patient who progressed to respiratory failure after Middle East respiratory syndrome coronavirus (MERS-CoV) infection. He did not show a serological response after convalescent plasma (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 transfusion-related acute lung injury (TRALI). **(C)** A previously healthy 32-year-old male patient who progressed to respiratory failure and received extracorporeal membrane oxygenation (ECMO) support after MERS-CoV infection. The donor plasma had neutralization activity with plaque reduction neutralization test (PRNT) titre 1:80, and the patient showed seroconversion the day after the CP infusion. IFA, immunofluorescence assay; Ig, immunoglobulin; MV, mechanical ventilation.

Figure 1. Continued

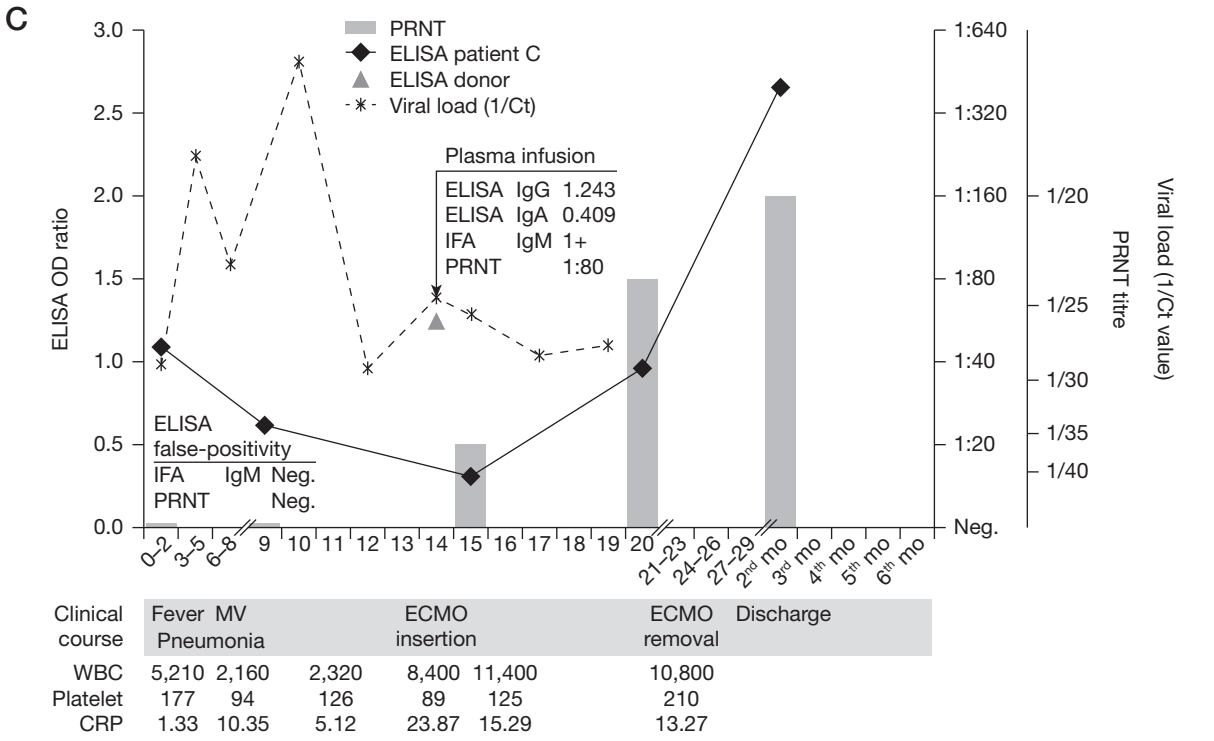


Table 1. Performance of anti-MERS-CoV ELISA IgG in predicting PRNT titre ≥1:80 in 138 MERS-CoV-infected patients

Predictive values	Cutoff 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 optical density (OD) ratio cutoffs. Area under the curve calculated by the receiver operating characteristic curve was 0.958. ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; MERS-CoV, Middle East respiratory syndrome coronavirus; NPV, negative predictive value; PPV, positive predictive value; PRNT, plaque reduction neutralization test.

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 aetiology 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 MERS-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 pneumonia patients showed seroconversion [12]. This difference suggests that donors should be tested for antibody titres, otherwise be selected from patients who recovered from severe illness.

Second, only patient C, who received donor plasma with a PRNT titre 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 titre of 1:40, and seroconversion occurred on dpoi 19, the median timing [12]. Although the seroconversion in patient C might not be related to 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 titre of 1:80 to achieve a meaningful serological 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 the neutralization test for MERS-CoV requires 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 titre ≥1:80. With an ELISA IgG cutoff value of OD ratio 1.6, ELISA IgG could predict PRNT titre ≥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, these results are limited with regard to evaluating the treatment's effectiveness, since only one infusion probably provoked a meaningful serological response. Other CP infusion performed in others centres 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 titre ≥1:80 might be required. ELISA IgG could substitute for neutralization tests in resource-limited situations.

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Disclosure statement

The authors declare no competing interests.

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