

# Convalescent Plasma Treatment Reduced Mortality in Patients With Severe Pandemic Influenza A (H1N1) 2009 Virus Infection

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**Background.** Experience from treating patients with Spanish influenza and influenza A(H5N1) suggested that convalescent plasma therapy might be beneficial. However, its efficacy in patients with severe pandemic influenza A(H1N1) 2009 virus (H1N1 2009) infection remained unknown.

**Methods.** During the period from 1 September 2009 through 30 June 2010, we conducted a prospective cohort study by recruiting patients aged  $\geq 18$  years with severe H1N1 2009 infection requiring intensive care. Patients were offered treatment with convalescent plasma with a neutralizing antibody titer of  $\geq 1:160$ , harvested by apheresis from patients recovering from H1N1 2009 infection. Clinical outcome was compared with that of patients who declined plasma treatment as the untreated controls.

**Results.** Ninety-three patients with severe H1N1 2009 infection requiring intensive care were recruited. Twenty patients (21.5%) received plasma treatment. The treatment and control groups were matched by age, sex, and disease severity scores. Mortality in the treatment group was significantly lower than in the nontreatment group (20.0% vs 54.8%;  $P = .01$ ). Multivariate analysis showed that plasma treatment reduced mortality (odds ratio [OR], .20; 95% confidence interval [CI], .06–.69;  $P = .011$ ), whereas complication of acute renal failure was independently associated with death (OR, 3.79; 95% CI, 1.15–12.4;  $P = .028$ ). Subgroup analysis of 44 patients with serial respiratory tract viral load and cytokine level demonstrated that plasma treatment was associated with significantly lower day 3, 5, and 7 viral load, compared with the control group ( $P < .05$ ). The corresponding temporal levels of interleukin 6, interleukin 10, and tumor necrosis factor  $\alpha$  ( $P < .05$ ) were also lower in the treatment group.

**Conclusions.** Treatment of severe H1N1 2009 infection with convalescent plasma reduced respiratory tract viral load, serum cytokine response, and mortality.

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Epidemiological studies showed that the pandemic influenza A(H1N1) 2009 virus (H1N1 2009) is similar to the seasonal influenza virus in many aspects including length of hospitalization, intensive care unit (ICU) admission, and frequency of deaths [1–3]. Ten to forty-four percent of the hospitalized patients required intensive care, with 25%–50% of these patients

eventually succumbing to death [1–8]. Patients who presented with severe disease were significantly younger and had good past health in comparison with patients with seasonal influenza [1–10]. This phenomenon was attributed to the absence of preexisting cross-reactive antibodies against this novel virus in patients born after 1950 [11]. Besides intensive care support [6], intravenous antiviral treatment [12, 13] and the application of extracorporeal membrane oxygenation (ECMO) in these patients were considered in view of their high mortality rate [14]. However, the efficacy of the intravenous antivirals was not well documented for severe influenza and the availability of ECMO is limited to only some tertiary hospitals. Convalescent plasma and hyperimmune intravenous immunoglobulin are the standard of care [15] for Argentine hemorrhagic fever caused by Junin virus [16] and red blood cell aplasia due to parvovirus B19 [17], whereas their usefulness in Ebola virus infection, Lassa fever, and severe acute respiratory syndrome (SARS) were uncertain [15, 18]. Meta-analysis of reports from the 1918 influenza A(H1N1) pandemic [19] and reports on the treatment of severe influenza A(H5N1) virus infection [20, 21] suggested that convalescent plasma might be an effective treatment option for patients with severe H1N1 2009 infection. Patients who have recovered from this infection with a high neutralizing antibody titer (NAT) provided a valuable source of the convalescent plasma and the absence of neutralizing antibody against this novel virus in the general population provided a unique opportunity to study its effect in the treatment of severe cases [22].

## METHODS

All patients aged  $\geq 18$  years with severe H1N1 2009 infection admitted to the ICUs of 7 hospital clusters under the Hospital Authority of Hong Kong from 1 September 2009 through 30 June 2010 were enrolled into a prospective cohort study, which was approved by the institutional review board of the Hospital Authority. Patients with severe H1N1 2009 infection were given the convalescent plasma treatment if they fulfilled the following criteria: adult patients aged  $\geq 18$  years with written informed consent given by the patient or next-of-kin, a laboratory-confirmed diagnosis of H1N1 2009 infection by positive reverse-transcription polymerase chain reaction (RT-PCR) testing of respiratory specimens [23, 24], and clinical deterioration despite optimal antiviral treatment that required intensive care within 7 days of symptom onset. Patients were excluded if they were aged  $< 18$  years, were hypersensitive to immunoglobulin, were known to have immunoglobulin A deficiency, or declined plasma treatment. Patients who declined plasma treatment were recruited as controls with verbal informed consent. Five hundred milliliters of convalescent plasma with NAT

of  $\geq 1:160$  was infused intravenously to patients in the treatment group over a period of 4 h.

As described elsewhere [25, 26], during the period from September through October 2009, patients who had recovered from H1N1 2009 infection were invited by the Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) to give informed consent for donation of their convalescent plasma voluntarily to treat patients with severe disease due to this novel virus. All potential donors had the diagnosis of H1N1 2009 infection confirmed by positive RT-PCR testing of the influenza A virus M and pandemic H1 genes and negative RT-PCR testing of the seasonal influenza A virus H1 and H3 genes in nasopharyngeal specimens. All donors had clinically recovered from the infection for at least 2 weeks and met the current HKRCBTS blood donor eligibility criteria for blood and plasma donation, including weight of  $> 50$  kg and NAT to H1N1 2009 of  $\geq 1:40$ , and successfully passed the infectious disease marker testing for negativity to hepatitis B surface antigen, hepatitis C virus antibody, human immunodeficiency virus antibody, and syphilis in their serum samples [25]. Upon donation, 500 mL of convalescent plasma was obtained from each donor by apheresis; plasma was frozen at  $-40^{\circ}\text{C}$  until usage. In this study, we used only convalescent plasma with NAT of  $\geq 1:160$  to treat patients with severe H1N1 2009 infection.

Clinical information of all enrolled patients was retrieved from the hospital computer medical system (CMS). This included the baseline demographic data, days of admission from symptom onset, and presenting symptoms. The Charlson index of comorbidity and Acute Physiology and Chronic Health Evaluation II (APACHE II) score upon ICU admission were estimated. Bacterial and viral co-infection was demonstrated by a positive culture from respiratory, urinary, blood or cerebrospinal fluid culture within 48 h of hospital admission. Complications including acute renal failure, acute coronary syndrome, myocarditis, acute respiratory distress syndrome, and nosocomial infection were recorded. Assisted ventilation with mechanical or bilevel positive airway pressure and the application of ECMO were recorded. Antiviral treatment including oral oseltamivir, intravenous peramivir or zanamivir, inhaled zanamivir, stress dose of intravenous hydrocortisone, antioxidant treatment with *N*-acetylcysteine, and convalescent plasma treatment was recorded (see the Appendix, which appears only in the online version of the *Journal*).

Nasopharyngeal specimens collected during ICU hospitalization were sent to the laboratory in viral transport medium. Total nucleic acid extraction was performed using a NucliSens easyMAG instrument (bioMerieux). RT-PCR was performed and viral load was determined in a blinded fashion, as described elsewhere [23, 27]. All procedures involving clinical specimens and influenza virus were performed in a biosafety level 2 laboratory with biosafety level 3 practices. The host immunological

response was monitored by the Luminex enzyme immunoassay (Luminex) for 25 different plasma cytokines and chemokines, as reported elsewhere [1].

Clinical and laboratory parameters were compared by the  $\chi^2$  test for categorical variables and the Mann-Whitney *U* test for continuous variables. Significant risk factors for death were further analyzed by multiple logistic regressions. Subgroup comparison by the Mann-Witney *U* test was performed for patients with serial viral load and cytokine level. SPSS for Windows (version 16.0; SPSS) was used for statistical computation. A *P* value of  $<.05$  was considered to represent significant difference.

## RESULTS

A total of 93 patients with severe H1N1 2009 infection were enrolled (Table 1). Twenty patients (21.5%) received the convalescent plasma treatment. Apart from more obese patients in the treatment group, both groups were matched by age, sex, Charlson index, and APACHE II score upon ICU admission. However, the treatment group had significantly fewer deaths (20% vs 54.8%; *P* = .01) and a lower median lymphocyte count upon ICU admission when compared with the control group. None of the patients in the treatment group developed adverse events from the convalescent plasma. Seventeen (85%) of the 500-mL convalescent plasma samples had NAT of 1:160 and 3 (15%) had NAT of 1:320.

Fifty-three patients were male, and the overall median age was 53.5 years (interquartile range [IQR], 41.5–59.5 years). The median interval from symptom onset to ICU admission was 3 days (IQR, 2–5 days). Most patients had few underlying diseases, with a median Charlson index of 1 (IQR, 0–1) and a median APACHE II score of 13 (IQR, 9–16). A majority (93.5%) of the patients required mechanical ventilation. Thirteen patients (14%) presented with bacterial co-infections upon admission. There was no difference in treatment, antiviral therapy, and rate of complications between the treatment and control groups (Table 1). Twenty-five patients were diagnosed with ventilator-associated pneumonia (see the Appendix, which appears only in the online version of the *Journal*).

Forty-four (47.3%) of the recruited patients died (Table 2). Univariate analysis showed that the treatment with convalescent plasma and complication of acute renal failure were the 2 factors significantly different between patients who survived and those who died. Multivariate analysis showed that treatment with convalescent plasma reduced mortality (odds ratio [OR], .20; 95% confidence interval [CI], .06–.69; *P* = .011), whereas the complication of acute renal failure was independently associated with death (OR, 3.79; 95% CI, 1.15–12.4; *P* = .028).

Subgroup analysis of 44 patients with serial viral load and cytokine level demonstrated that there were no significant differences in the initial viral load and cytokine level between the 2

groups on the first day of ICU admission (Table 3). Convalescent plasma was infused on day 2 (median; IQR, day 1–2.5) of ICU admission. Subsequent viral loads measured on day 3, 5, and 7 after ICU admission were significantly lower in the treatment than in the control group (*P* < .001, *P* = .02, and *P* = .04, respectively). The corresponding day 5 interleukin 6 (IL-6; *P* = .02), day 5 interleukin 10 (IL-10; *P* < .01), day 5 tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ; *P* = .02), day 7 IL-10 (*P* = .01), and day 9 IL-10 (*P* = .03) levels were lower in the treatment group compared with the control group. Overall, the viral load in the treatment group decreased at a higher rate than that in the control group, with the maximal difference on day 7 after ICU admission (viral load, 1.5 log<sub>10</sub> copies/mL). The IL-6, IL-10, and TNF- $\alpha$  response lagged behind the viral load reduction by 2 days in the treatment group, which then decreased rapidly (Figures 1–3). The maximal difference in IL-6, IL-10, and TNF- $\alpha$  level between the 2 groups occurred on day 9 (1.16 log<sub>10</sub> pg/mL), day 3 (.61 log<sub>10</sub> pg/mL), and day 5 (.26 log<sub>10</sub> pg/dL), respectively.

## DISCUSSION

Although the majority of patients infected by pandemic influenza A(H1N1) 2009 virus had a mild illness, severe diseases and mortality occurred in those with extremes of age, immunosuppression, obesity, pregnancy, and other underlying illnesses [1, 6]. Patients with mild illness fared well with the early use of neuraminidase inhibitors [9, 24]. However, the usefulness of these antiviral agents in treating patients with severe illness is uncertain. Despite the use of double-dose oseltamivir and inhaled zanamivir, patients with severe illness had delayed clearance of viral load in respiratory secretions, associated with persistent elevation of cytokines in their serum samples [1]. Thus, there is an urgent need to find alternative therapeutic regimens for managing this subgroup of patients. Robust protection from lethality for at least 72 h after infection was demonstrated for monoclonal antibodies with neutralizing activity produced by immortalized B lymphocytes of convalescent patients recovering from influenza A(H5N1) virus infection in a murine model challenged by the hypervirulent influenza A(H5N1) virus [28]. Furthermore, meta-analysis on studies using convalescent blood products in the 1918 influenza pandemic suggested that such an approach could reduced the mortality rate of severe cases by >50% [19]. Therefore, a prospective multicenter case-control study was conducted. Our findings suggested that 1 dose of convalescent plasma with NAT of  $\geq 1:160$  was effective in reducing mortality, respiratory tract viral load, and serum level of cytokines. This is not unexpected because 1 critically ill patient with hyperinflammatory avian influenza A(H5N1) virus infection had rapidly responded to 600 mL of convalescent plasma with NAT of 1:80 [20]. The radiological consolidation improved, and

**Table 1. Comparison of Demographic and Clinical Factors Between the Treatment (Convalescent Plasma) Group and the Control (Non-Convalescent Plasma) Group**

Demographic or clinical factors	Convalescent plasma treatment		P
	Yes (n = 20)	No (n = 73)	
Male/female	11/9 (55/45)	53/20 (72.6/27.4)	.17
Age, years, median (IQR)	48 (37–55.8)	54 (43–61.5)	.06
Days of admission from symptom onset, median (IQR)	3.5 (3–4.8)	3 (2–5)	.25
Smoker	3 (15)	17 (23.3)	.55
Obesity (BMI of >27) <sup>a</sup>	7 (14)	9 (12.3)	.04
Charlson index of comorbidity, median (IQR)	0 (0–1)	0 (0–1)	.54
APACHE II score upon ICU admission, median (IQR) <sup>a</sup>	12 (8–15)	13 (9–16)	.39
Bacterial co-infection	3 (15)	10 (13.7)	1.00
<b>Symptoms</b>			
Fever	19 (95)	55 (75.3)	.06
Cough	20 (100)	57 (78.1)	.02
Sputum	15 (75)	53 (72.6)	1.0
Dyspnea	20 (100)	59 (80.8)	.04
Diarrhea	1 (5)	4 (5.5)	1.0
<b>Complications</b>			
Nosocomial infection	3 (15)	22 (30.1)	.26
Myocarditis	1 (5)	8 (11)	.68
Acute coronary syndrome	0 (0)	9 (12.3)	.20
Acute renal failure	3 (15)	15 (20.5)	.75
ARDS	14 (68)	38 (52.1)	.21
<b>Treatment</b>			
Mechanical ventilation	18 (90)	69 (94.5)	.61
BiPAP ventilation	4 (20)	6 (8.2)	.11
Application of ECMO	4 (20)	8 (11)	.28
Stress steroid treatment	9 (45)	29 (40)	.80
N-acetylcysteine treatment	4 (20)	18 (25)	.77
<b>Antiviral treatment</b>			
Oseltamivir	20 (100)	70 (95.9)	1.00
Inhaled zanamivir	8 (42.1)	22 (30.1)	.43
Intravenous zanamivir	2 (10)	3 (4.1)	.27
<b>Laboratory parameters upon ICU admission</b>			
Hemoglobin level, 10 <sup>9</sup> /L, median (IQR)	12.4 (11.4–14)	13.1 (10.6–14.6)	.72
Neutrophil count, 10 <sup>9</sup> /L, median (IQR)	5.2 (2.8–10.8)	6.7 (4.6–11.4)	.12
Lymphocyte count, 10 <sup>9</sup> /L, median (IQR)	.6 (.4–.7)	.7 (.5–1.2)	.02
Platelets, 10 <sup>9</sup> /L, median (IQR)	133 (108–180)	162 (118–225)	.15
ALT level, U/L, median (IQR)	34 (28–48)	41 (20–76)	.86
Creatinine level, μmol/L, median (IQR)	88 (58–107)	97 (71–177)	.08
<b>Outcome</b>			
Death	4 (20)	40 (54.8)	.01

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. ALT, alanine transaminase; APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; BiPAP, bilevel positive airway pressure; BMI, body mass index; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IQR, interquartile range. Reference ranges are as follows: hemoglobin level, 12.4–16.8 g/dL; lymphocyte count, 1.2–3.4 × 10<sup>9</sup>/L; neutrophil count, 2.2–6.7 × 10<sup>9</sup>/L; ALT level, 5–31 U/L; and creatinine level, 66–106 μmol/L.

<sup>a</sup> Eight patients did not have their body weight measured and 6 patients did not have their APACHE II score estimated.

the respiratory tract viral load decreased by >3 log<sub>10</sub> copies/mL within 48 h after plasma therapy. Although the convalescent plasma may contain other viral neutralizing antibodies or opsonising antibodies bacterial pathogens, which might decrease the severity of either coexisting community-acquired or

subsequent hospital-acquired infections, no viral co-infection upon admission or significant reduction in hospital-acquired infection was observed in the present study. Nevertheless, we cannot exclude the possibility that the convalescent plasma therapy might have reduced the harmful effect of nondetected

**Table 2. Comparison of Demographic, Clinical, and Laboratory Factors With Outcome of 93 Patients**

Demographic and clinical Factors	Clinical outcome		P
	Survived(n = 49)	Died(n = 44)	
Male sex	31 (63)	33 (75)	.27
Age, years, median (IQR)	52 (38.5–58)	55 (44.3–60)	.32
Days of admission from symptom onset, median (IQR)	3 (2.5–5)	3 (2–4.8)	.41
Smoker	12 (24)	8 (18.2)	.61
Obesity (BMI of >27) <sup>a</sup>	9 (18.9)	7 (15.9)	.79
Charlson index of comorbidity, median (IQR)	0 (0–1)	0 (0–1)	.56
APACHE II score upon ICU admission, median (IQR) <sup>a</sup>	11 (8–14)	14.5 (10.3–16)	.10
Bacterial co-infection	4 (8.2)	9 (20.5)	.13
<b>Symptoms</b>			
Fever	41 (83.7)	33 (75)	.32
Cough	43 (87.8)	34 (77.3)	.27
Sputum	35 (71.4)	33 (75)	.82
Dyspnea	41 (83.7)	38 (86.4)	.78
Diarrhea	2 (4.1)	3 (6.8)	.67
<b>Complications</b>			
Nosocomial infection	16 (32.7)	9 (20.5)	.24
Myocarditis	3 (6.1)	6 (13.6)	.30
Acute coronary syndrome	3 (6.1)	6 (13.6)	.30
Acute renal failure	5 (10.2)	13 (29.5)	.03
ARDS	29 (59.2)	23 (52.3)	.54
<b>Treatment</b>			
Mechanical ventilation	46 (93.9)	41 (93.2)	1.0
BiPAP ventilation	5 (10.2)	5 (11.4)	1.0
Application of ECMO	9 (18.4)	3 (6.8)	.13
Convalescent plasma treatment	16 (32.7)	4 (9.1)	.01
Stress steroid treatment	20 (40.8)	18 (40.9)	1.0
N-acetylcysteine treatment	15 (30.6)	7 (15.9)	.14
<b>Antiviral treatment</b>			
Oseltamivir	49 (100)	41 (93.2)	.10
Inhaled zanamivir	17 (34.7)	13 (29.5)	.66
Intravenous zanamivir	2 (4.1)	3 (6.8)	.67
<b>Laboratory parameters upon ICU admission</b>			
Hemoglobin level, 10 <sup>9</sup> /L, median (IQR)	13.1 (11.3–14.3)	12.7 (10–14.8)	.77
Neutrophil count, 10 <sup>9</sup> /L, median (IQR)	6.6 (3.4–10.7)	8.2 (5.5–14.7)	.46
Lymphocyte count, 10 <sup>9</sup> /L, median (IQR)	.6 (.4–.9)	.7 (.5–1.1)	.70
Platelet count, 10 <sup>9</sup> /L, median (IQR)	168 (121.5–221)	131.5 (112.3–215.8)	.21
ALT level, U/L, median (IQR)	42 (23–71.5)	34 (20.5–78)	.76
Creatinine level, μmol/L, median (IQR)	90 (59–144)	97.5 (72–184)	.13

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. ALT, alanine transaminase; APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; BiPAP, bilevel positive airway pressure; BMI, body mass index; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IQR, interquartile range. Reference ranges are as follows: hemoglobin level, 12.4–16.8 g/dL; lymphocyte count, 1.2–3.4 × 10<sup>9</sup>/L; neutrophil count, 2.2–6.7 × 10<sup>9</sup>/L; ALT level, 5–31 U/L; and creatinine level, 66–106 μmol/L. This table appears only in the online version of the *Journal*.

<sup>a</sup> Eight patients did not have their body weight measured and 6 patients did not have their APACHE II score estimated.

coexisting community-acquired infection in the treated patients and therefore decreased the cytokine level and mortality. Although this is not a randomized placebo-controlled trial, it is important to demonstrate that the treatment group had lower mortality despite having more risk factors associated with disease severity, including a lower lymphocyte count, more patients with obesity, and presentation with more severe symptoms.

Another observation not reported in previous studies is the development of acute renal failure being another independently significant risk factor associated with mortality. This is not unexpected as marked increases in cytokine and chemokine levels in these severe cases are often associated with multiorgan dysfunction, vascular thrombosis, lymphoid atrophy, and reactive hemophagocytosis [1]. Renal failure is just one of the

**Table 3. Comparison of Viral Load and Cytokine Level Between Treatment (Convalescent Plasma) and Control (Non-Convalescent Plasma) Groups After Intensive Care Unit Admission in 44 Patients**

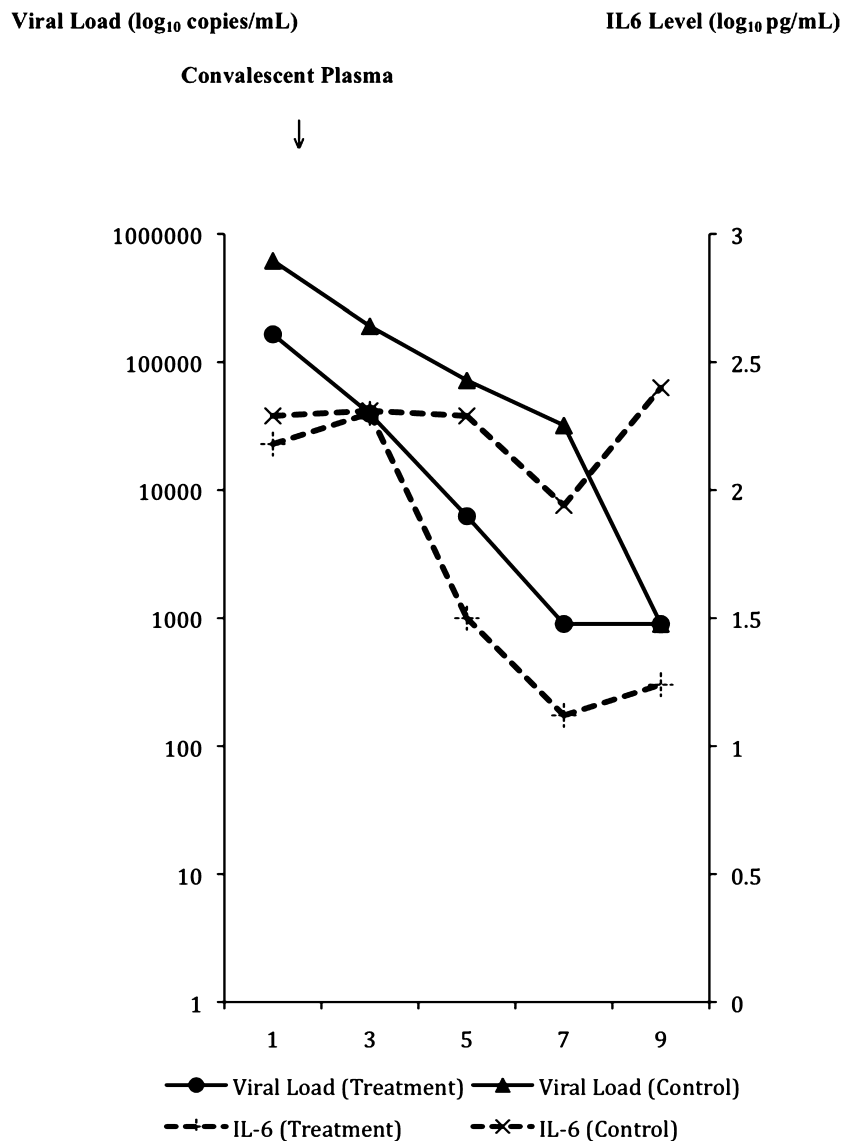
Median	Day 1	Day 3	Day 5	Day 7	Day 9
Viral load, copies/mL (samples available)	26	39	39	26	14
Treatment	1.65E5	3.95E4	6.25E3	900	900
Control	6.18E5	1.91E5	7.21E4	3.21E4	900
<i>P</i>	.43	<.001	.02	.04	.90
IFN- $\alpha$ 2 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	19	21
Treatment	1.76	1.35	1.10	.45	.79
Control	1.07	.45	.61	.20	.88
<i>P</i>	.05	.05	.53	.30	.56
IL-1 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	2.31	2.04	1.95	1.67	1.83
Control	2.11	1.99	1.83	1.76	2.04
<i>P</i>	.32	>.999	.53	.90	.39
IL-6 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	2.18	2.30	1.50	1.12	1.24
Control	2.29	2.31	2.29	1.94	2.40
<i>P</i>	.76	.24	.02	.13	.11
IL-8 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	1.69	1.78	1.59	1.73	1.86
Control	2.19	2.24	2.02	2.03	2.10
<i>P</i>	.11	.50	.81	.43	.39
IL-10 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	1.45	1.25	.80	.93	1.48
Control	1.80	1.86	1.35	1.12	1.74
<i>P</i>	.27	.12	<.01	.01	.03
IP-10 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	4.00	3.56	3.39	3.00	3.07
Control	3.60	3.49	3.37	3.02	3.82
<i>P</i>	.14	.58	.48	.71	.25
MCP-1 concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	2.86	2.93	2.59	2.47	2.87
Control	3.37	3.51	2.87	2.79	3.01
<i>P</i>	.35	.07	.17	.26	.44
G-CSF concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	1.47	1.57	1.12	.53	.20
Control	1.82	1.79	1.42	.20	.70
<i>P</i>	.24	.29	.47	>.999	>.999
TNF- $\alpha$ concentration, log <sub>10</sub> pg/mL (samples available)	28	22	29	20	21
Treatment	1.08	.97	.92	.95	.85
Control	1.24	1.32	1.18	1.28	1.06
<i>P</i>	.17	.11	.02	.12	.17

**NOTE.** The lowest detection limit of viral load was 900 copies/mL. The treatment group had 17 patients, and the control group had 27 patients. Convalescent plasma was infused on day 2 (median) of intensive care unit admission. G-CSF, granulocyte colony-stimulating factor; IFN- $\alpha$ 2, interferon  $\alpha$ 2; IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IP-10, interferon- $\gamma$  inducible protein-10; MCP-1, monocyte chemoattractant protein 1; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ . This table appears only in the online version of the *Journal*.

more readily quantifiable markers of multiorgan dysfunction. A similar finding has also been reported in large case series of infection by SARS-associated coronavirus [29].

It is also reassuring to see that no patients suffered from any adverse effect from the transfusion of just 500 mL of convalescent fresh frozen plasma. The NAT in patients was not measured

because an epidemiological study performed by our center demonstrated that only 3.3% of individuals aged 5–59 years in Hong Kong had NAT of  $\geq 1:40$  at the early phase of the epidemic [30]. All the plasma donors of this study had mild diseases, and therefore viremia would not be expected (unpublished data, Hung IF, To KK, Yuen KY, November 2009). Unlike the days in



**Figure 1.** Temporal changes of viral load and IL-6 level in treatment and control groups

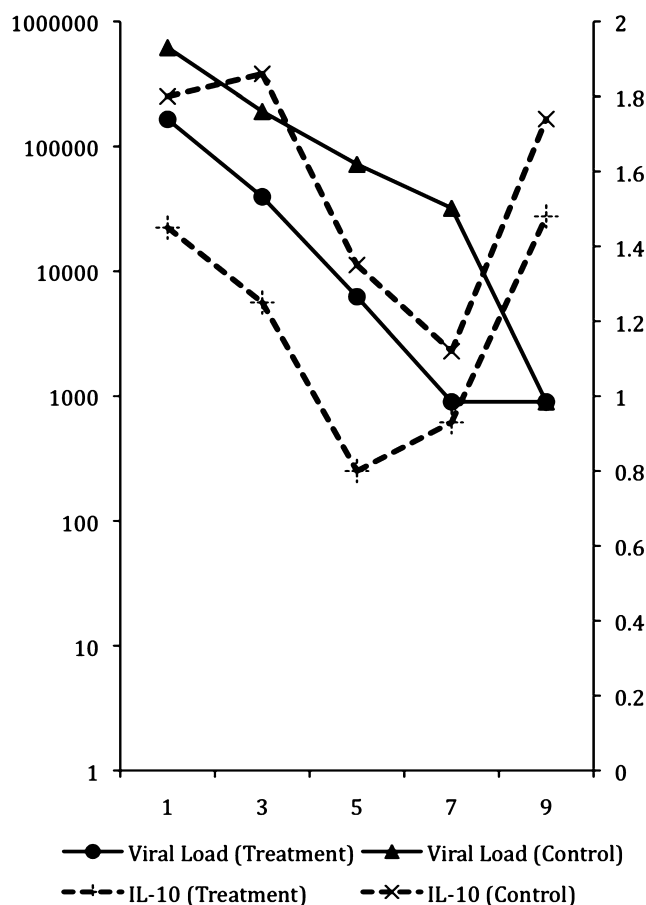
1918, donor screening, virological and microbiological testing, and apheresis have made such therapy safe and comfortable to both recipients and donors. The allowable plasma volume obtained by automated apheresis is 625 mL for donors with a body weight of 50–80 kg [31]. As the body weight of Chinese donors is more toward the lower limit, we routinely take 500 mL of plasma by apheresis. Theoretically 0.5 L of plasma with NAT of 1:320 diluted within a total interstitial fluid volume of ~15 L will give NAT of ~1:11. Results from our previous study on the level of neutralizing antibody against H1N1 2009 demonstrated that 10% of the convalescent donors had antibody titers of  $\leq 1:20$  [26]. Extrapolation from a vaccination study suggested that a geometric mean titer of hemagglutination inhibition antibodies of 1:40 and 1:20 would give ~99% and ~70% seroprotection at 1 month, respectively [32]. In the case report of influenza A(H5N1)

treated with convalescent plasma, 600 mL of plasma with NAT of 1:80 was sufficient to achieve a cure [20]. The typical volume of convalescent plasma or serum collected from a donor 7–60 days after resolution of symptoms in the 1918 pandemic was 125–250 mL given on 1 or 2 occasions [19]. Thus, the use of 500 mL with NAT of  $\geq 1:160$  would balance well between donor tolerability, volume overload, and sufficient antibody delivery in recipients.

When compared with antivirals, neutralizing antibodies do not suffer from the problem of drug resistance to neuraminidase inhibitors or adamantanes due to viral mutations, which can appear very rapidly [32–34]. Antigenic drift generally takes a few years and usually comes gradually. Therefore, significant antigenic drift leading to loss of effectiveness is unlikely to affect treatment efficacy within the timescale of this pandemic. Moreover, the polyclonal nature of neutralizing antibodies in

Viral Load ( $\log_{10}$  copies/mL)IL-10 Level ( $\log_{10}$  pg/mL)

Convalescent Plasma



**Figure 2.** Temporal changes of viral load and IL-10 level in treatment and control groups

convalescent plasma would minimize the risk of an escape mutant, which is more likely to emerge in patients treated with monoclonal antibody. The effect of convalescent plasma may be quite immediate in terminating the infective process and dampening down the cytokine response, whereas adamantanes and neuraminidase inhibitors or even newer antivirals active against the viral nucleoprotein [35] cannot neutralize any virus that have already entered the host cells. These drugs prevent either viral disassembly after viral entry or viral release from host cells. Although it is unclear how convalescent plasma improves the outcome of our patients, the neutralizing and nonneutralizing antibody in the plasma may also facilitate viral entry into Fc-receptor-bearing antigen-presenting cells such as macrophages and B lymphocytes. Since these cells are generally not permissive to the growth of influenza virus [36], increased viral uptake is unlikely to

compromise cell function but may actually increase viral antigen processing and presentation to augment T lymphocyte-mediated adaptive immune responses. [37].

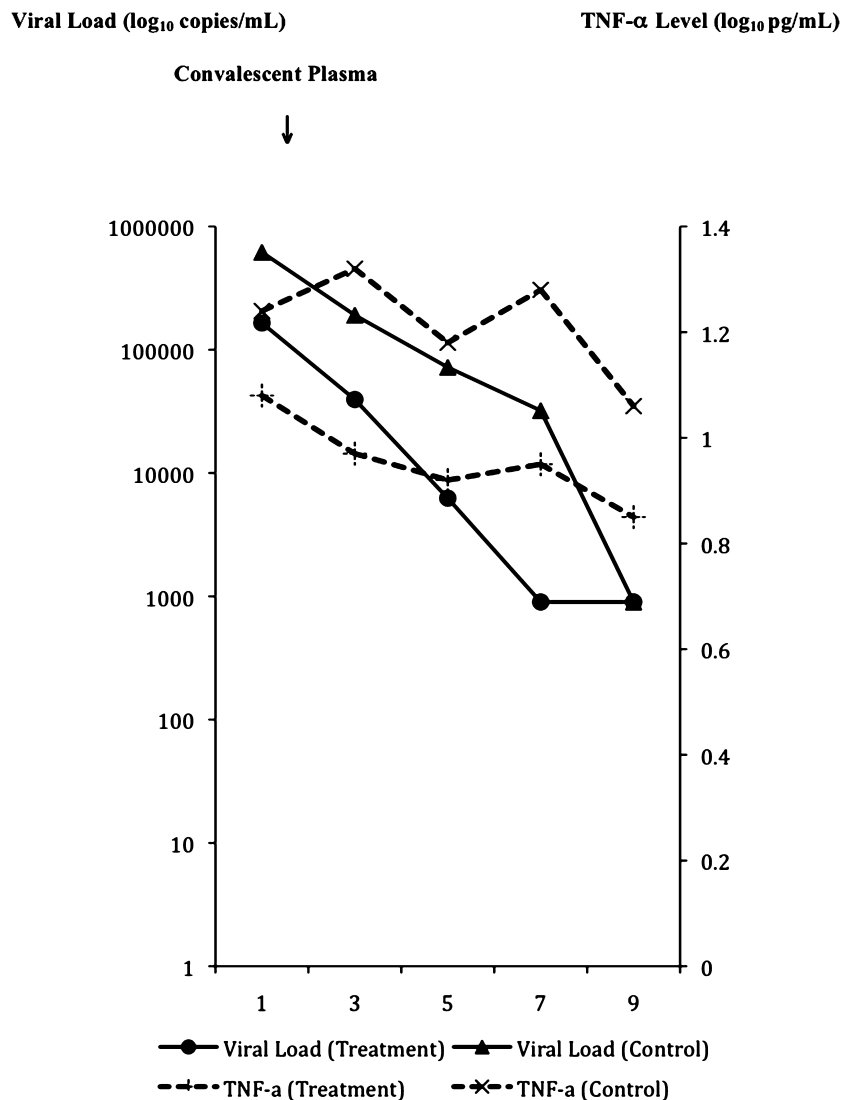
In conclusion, this study has demonstrated that convalescent plasma treatment may have a place in the treatment of patients with severe H1N1 2009 infection. The treatment effectively reduced the viral load and dampened the cytokine response with reduced mortality. A double-blind randomized controlled trial with hyperimmune intravenous immunoglobulin on patients with severe influenza infection is warranted in the future.

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**Figure 3.** Temporal changes of viral load and TNF- $\alpha$  level in treatment and control groups

Food and Health Bureau; and the Hospital Authority of the Hong Kong Special Administrative Region.

**Potential conflicts of interest.** none reported.

## APPENDIX

Baseline demographics included past medical and smoking history and obesity (defined as a body mass index of  $>27$ ). Presenting symptoms included fever (defined as a tympanic temperature of  $>37.8^{\circ}\text{C}$ ), cough, sputum production, sore throat, rhinorrhea, and shortness of breath.

Respiratory tract samples collected upon admission to the ICU were also assessed by multiplex PCR (Luminex) with a ResPlex II assay (version 2.0; Qiagen) for co-infection with respiratory syncytial virus, influenza B virus, parainfluenza viruses 1–4, human metapneumovirus, enteroviruses, rhinovirus, adenovirus, bocavirus, and coronaviruses NL63, HKU1, 229E, and OC43, in accordance with manufacturer's instructions.

Acute renal failure was defined as a serum creatinine level of  $>106\text{ }\mu\text{mol/L}$  or an increase in serum creatinine of  $\geq 50\%$  over a period of 48 h. Both myocarditis and acute coronary syndrome were diagnosed on the basis of electrocardiographic results and an elevated myocardial enzyme level (troponin or creatine kinase), with cardiac magnetic resonance imaging if indicated. Acute respiratory distress syndrome was defined as acute onset of respiratory failure, with an arterial oxygen partial pressure/inspired oxygen concentration relationship ( $\text{Pao}_2/\text{Fio}_2$ ) of  $\leq 200\text{ mm Hg}$  and bilateral infiltrates on chest radiograph, in the absence of a wedge pressure of  $>18\text{ mm Hg}$  or clinical heart failure. Nosocomial infection was demonstrated by a positive culture from respiratory, urinary, blood, or cerebrospinal fluid culture after 48 h of hospital admission until discharge from the ICU.

Positive sputum culture was found in samples from 4 patients with methicillin-sensitive *Staphylococcus aureus*, 2 patients with *Streptococcus pneumoniae*, 2 patients with community-associated methicillin-resistant *S. aureus*, 2 patients with

*Pseudomonas aeruginosa*, 1 patient with *Haemophilus influenzae*, and 1 patient with *Mycobacterium tuberculosis*. One patient had a positive blood and urine culture with *Escherichia coli*. None of the patients had a viral co-infection. Of the 25 patients diagnosed ventilator-associated pneumonia, endotracheal aspirate culture was positive in samples from 7 patients with *Acinetobacter baumannii*, 7 patients with *P. aeruginosa*, 4 patients with *Klebsiella pneumoniae*, 2 patients with *Stenotrophomonas maltophilia*, 1 patient with *Citrobacter* species, 2 patients with *Enterococcus faecalis*, and 2 patients with *Enterobacter* species. Another 2 patients had blood culture positive for *E. coli*.

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