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Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital

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***Objectives*: To describe the immunological responses and clinical outcome of coronavirus (SARS) infected healthcare workers (HCW) who had been administered with convalescent plasma as a treatment.**

***Methods*: Convalescent plasma (500 mL) was obtained from each of three SARS patients and transfused into the three infected HCW. Donors were blood type O and seronegative for hepatitis B and C, HIV, syphilis and human T-cell lymphotropic virus types I and II (HTLV-I and -II). Serum antibody (IgG) titre was >640. Apharesis was performed with a CS 3000 plus cell separator followed by the forming of the convalescent phase plasma. As part of the routine check with donated plasma, the convalescent plasma was confirmed free of residual SARS-CoV by RT–PCR. Serial serum samples obtained from the recipients of the convalescent plasma were collected to undertake real-time quantitative RT–PCR for SARS-CoV for direct measurement of viral concentration. Specific immunoglobulin IgM and IgG concentrations were titrated using an antigen microarray developed in-house.**

***Results*: Viral load dropped from 495×10^3, 76×10^3 or 650×10^3 copies/mL to zero or 1 copy/mL one day after transfusion. Anti-SARS-CoV IgM and IgG also increased in a time-dependent manner following transfusion. All three patients survived. One HCW became pregnant subsequently, delivering 13 months after discharge. Positive anti-SARS-CoV IgG was detected in the newborn. Passive transfer of anti-SARS-CoV antibody from the mother was considered as a possibility.**

***Conclusions*: All infected HCW whose condition had progressed severely and who had failed to respond to the available treatment, survived after transfusion with convalescent plasma.**

Keywords: SARS, coronavirus, therapy

Introduction

During outbreaks of severe acute respiratory syndrome (SARS), healthcare workers (HCW) are at high risk of infection due to the various clinical presentations of infected patients and/or the close contact care.[1] Although much has been learned about the clinical features of the disease as well as the characteristics of the causative agent, coronavirus,[2–4] treatment remains controversial. Since the SARS epidemic, numerous articles have been published describing the clinical features and management of SARS. Suggested

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treatments, including ribavirin and methylprednisolone therapy, according to immediate patient response have also been documented.[5] Unfortunately, lack of clinical proof has prevented confirmation of these therapies, and *in vitro* experiments to determine inhibitory concentrations of ribavirin do not support the achievement dose due to the limitations imposed by cellular toxicity in the human body.[6] Although this life-threatening disease appears to be under control following major worldwide efforts and implementation of quarantine policy, the return of SARS is possible. Although drugs with anti-coronavirus (CoV) activities have been identified,[7–11] as yet no anti-CoV drug has been approved and a vaccine has yet to be made available. Previous reports on other viral infections have suggested that convalescent plasma or serum is effective where no other treatment is available or in an emergency.[12,13]

In this report, we present our experience with convalescent plasma as a treatment alternative, where no clinical improvement had been observed using standard treatment in SARS-infected HCW.

Methods and materials

Patients and HCW in SARS isolation wards

On 22 April 2003, a major outbreak of SARS was declared at Taipei Municipal Hoping Hospital (TMHH), with a large number of suspected and probable cases subsequently transferred and redistributed to different hospitals for quarantine and further medical care. Before this transfer from TMHH, the Tri-service General Hospital (TSGH) had admitted 18 suspect cases to its emergency department, and 20 patients were then transferred from TMHH within the next 2 weeks. A total of 157 suspect or probable SARS cases were admitted during this outbreak, with a total of 580 HCW (160 physicians and 420 nurses) involved in patient care in the SARS isolation ward. During the SARS period at TSGH, five confirmed cases from the TMHH developed respiratory failure after admission, with two ultimately expiring.

Serology test and RT–PCR detection of coronavirus

Anti-CoV IgG antibody was detected by indirect immunofluorescence assay using Vero cells infected with a strain of SARS-CoV (GenBank accession no. AY278554) isolated from a SARS patient. Screening tests were performed as described previously.[14] Serum specimens were tested at a dilution of 1:40, and, if positive at titers ≥40, the testing was repeated and specimens were titrated to the endpoint using 2-fold serial dilutions. Where the titre was ≥40, a second test of the serum samples was performed, and all of the previously obtained serum samples were also examined for SARS-CoV IgG. Serum from HCW who had been diagnosed as probable SARS cases or who were in close contact with them was blinded and sent to the Department of Microbiology, The Chinese University of Hong Kong, for further confirmation.

The primers and probes used for the RT–PCR SARS-CoV detection were synthesized according to the recommendations of the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA).[15] The viral RNA from the throat-swab specimens was extracted using the MagNA Pure LC total nucleic acid isolation kit (Roche, Mannheim, Germany). After extraction, 5 μL of the RNA extract was used as the template for all PCR assays in 50 μL reaction volumes containing 10 μL of 5 × buffer, 2 μL of enzyme mix, 2 μL of deoxynucleoside triphosphate and 0.6 μM each of sense and antisense primers. The reaction was subjected to a precycle consisting of 50°C for 30 min and 95°C for 15 min. Forty cycles of amplification were then conducted at 95°C for 30 s, 50°C for 40 s and 72°C for 1 min. For real-time quantitative RT–PCR assays, a 20 μL reaction volume containing 12 μL of human pneumonia associated (HPA)-Coronavirus LC Master mix, 3 μL of HPA-Coronavirus LC Mg-Sol and 0.5 μL of HPA-Coronavirus LC internal control were thermal-cycled using a Light Cycler (Roche) as follows: 50°C for 10 min for RT reaction, 95°C for 10 min for denaturation, followed by 45 cycles of amplification each of which consisted of 95°C for 2 s, 55°C for 12 s and 72°C for 10 s.

Donors and HCW selected for convalescent plasma transfusion

Two HCW and one laboratory researcher who became infected as a result of their involvement in SARS patient care were selected for convalescent plasma transfusion after failure of empirical treatment using ribavirin and methylprednisolone. Before commencement, emergency approval for the convalescent plasma transfusion as a clinical trial was obtained from the Department of Health. Following informed consent, convalescent plasma was obtained from a married couple and another patient: one was the index case of the TMHH outbreak, and the others had recovered at day 47 and 3 months, respectively, after SARS-CoV infection. Donors were blood type O and seronegative for hepatitis B and C, HIV, syphilis and human T-cell lymphotropic virus types I and II (HTLV-I and -II). Serum antibody (IgG) titre was >640. Apharesis was performed with a CS 3000 plus cell separator (Baxter, Deerfield, IL, USA) followed by the formation of the convalescent phase plasma. As part of the routine check with plasma donation, the convalescent plasma was also confirmed free of residual SARS-CoV by RT–PCR.

Virus load and antibody determination following convalescent plasma transfusion

Serial serum samples obtained from the recipients of the convalescent plasma were collected to conduct real-time quantitative RT–PCR for SARS-CoV in order to measure viral concentration directly as previously described.[16] Specific immunoglobulin IgM and IgG concentrations were titrated using an antigen microarray developed in-house. Briefly, the purified recombinant nucleocapsid protein of SARS-CoV at different concentrations was printed on glass microscope slides using computer-controlled high-speed robotics (Microsys™; Cartesian Technologies, CA, USA). The printed slides were incubated for 3 min at room temperature in a solution containing 2% (w/v) skimmed milk in phosphate-buffered saline (PBS) to block non-specific antibody binding.

Serum samples were diluted 1:100 in 2 × PBS containing 2% (w/v) skimmed milk and 0.1 mL/L Tween 20 and allowed to react with the array for 30 min at room temperature in a humid chamber. To reveal IgG binding to the printed antigens, the slides were washed five times (1 mL each) with PBS containing 0.1 mL/L Tween 20, and subsequently incubated for 30 min with Cy 5-labelled anti-human IgG monoclonal antibody suspended in a solution containing 2 × PBS, 2% (w/v) non-fat milk and 0.1 mL/L Tween 20. To reveal IgM, the slides were incubated for 30 min with Cy 5-labelled goat immunoglobulins directed against the anti-human IgM μ chain. The efficiency of the labelling procedure was assessed by measuring the molar ratio of dye to protein and analysing it with a scanner (Genepix 4000B; Axon, CA, USA). The quantities of IgG and IgM in the sera were determined by interpolating the photomultiplier counts collected at the recombinant nucleocapsid protein spots with the IgG and IgM internal calibration curves.

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Results

Characteristics of HCW with nosocomial SARS-CoV infection

The first suspected case of SARS-CoV infection in a HCW was observed in an intern on 3 May 2003. This 28-year-old male experienced fever to 38°C, but as there were no other signs of infection such as pneumonia, diarrhoea or leucopenia, he was initially self-quarantined in a separate dormitory. The fever subsided after 2 days, but rose to 37.8°C on 10 May 2003 and he was immediately admitted to an isolation ward. Cough, malaise, rhinorrhoea, sore throat and headache subsequently occurred and he was deemed to be a probable SARS case. During the quarantine in his dormitory, two nurses, who had been in direct care of SARS patients, were admitted to the isolation ward on 8 and 10 May 2003. They had signs of fever, myalgia and/or headache. An investigation into outbreaks among HCW was initiated and a total of 89 HCW were quarantined. Subsequently, fever was observed in another three nurses and a resident physician during the period 11–15 May (Table 1). Apart from HCW 6, a resident who requested further self-quarantine, three nurses were admitted immediately after experiencing fever. Most of these HCW had worked frequently on one of the negative pressure wards. One of the two negative pressure wards was then immediately closed on 16 May. All patients in the closed ward were then transferred to the remaining negative pressure ward and no more cases of fever were observed in the HCW thereafter. The last patient in the isolation ward (HCW 7) was discharged on 14 June. The specific isolation wards for SARS were closed down on the day the last patient was discharged. No SARS case was noted subsequently until 16 December 2003. A SARS-CoV researcher experienced fever on 10 December and was self-quarantined. As the fever had not subsided after 5 days, he was finally admitted as a probable SARS case. RT–PCR of the sputum was immediately performed on admission and the researcher was confirmed positive for CoV infection. Serum from all the above infected HCW was collected after 3–5 weeks. Serum IgG antibody titre ranged from 160 to >640 (Table 1).

Clinical features and response to empirical treatment

The most common symptoms at presentation were: fever with body temperature of >38.0°C (87.5%); cough and chills (37.5%); rhinorrhoea, sore throat and vomiting (25%). None of the HCW presented with diarrhoea or dyspnoea on admission; however, three of them developed these maladies after 4–15 days. At fever onset, four HCW had abnormal chest radiographs, with two increased lung markings and two patch infiltrations. Empirical treatment with oral ribavirin (1200 mg/day) and moxifloxacin (400 mg/day) were commenced on the day of admission for all HCW, but not the researcher. Methylprednisolone (2 mg/kg/day) and intravenous immunoglobulin G (IVIG) (1 mg/kg/day for 2 days) were given to HCW 4 and 7 after persistence of chest radiographic progression. Because of progression of pulmonary infiltration and oxygen desaturation, convalescent plasma was then transfused at a rate of 2 mL/min on days 11 and 10 after admission, respectively. In the laboratory-acquired case (HCW 8), protease inhibitor (lopinavir 400 mg/ritonavir 100 mg/12 h) with convalescent plasma was started after confirmation of SARS-CoV infection. Serial chest X-rays revealed progression of pulmonary infiltration in five HCW, becoming most severe at day 9 of the fever onset. All but one HCW experienced at least two fever peaks. Three fever peaks were observed in two HCW who failed to respond to the empirical treatment and developed severe progression.

Clinical response and microbiology investigation before and after convalescent plasma transfusion

Three HCW (4, 7 and 8) received convalescent plasma transfusion, two due to severe progression while the other was involved in a treatment trial. Serial chest radiographs showed progression of pulmonary infiltration in HCW 4 and 7, who had failed to respond to the empirical treatment. After 1 day of convalescent plasma transfusion, body temperature decreased from >38 to <37°C. Radiological improvement was also observed after the convalescent plasma transfusion. Pulmonary opacities were completely resolved in HCW 4 before discharge.

Real-time quantitative SARS-CoV RNA measurement of the blood samples revealed high viral loads of 495×10^3, 76×10^3 and 650×10^3 copies/mL, respectively, for HCW 4, 7 and 8, detected 1 h before convalescent plasma transfusion. Viral load was no longer detectable from sequential blood samples after 24 h of transfusion, although the detection limit for viral load assay was presumed to be <10 copies per assay. In contrast, low titres of IgM and IgG were observed in the initial samples before the transfusion but increased subsequently in a time-dependent manner (Table 2).

Table 1. Characteristics of health care workers with SARS-coronavirus infection

HCW	Sex	Age	Job title	Onset	Admission date	Principal symptoms	Exposure location	IgG Ab titre	PCR
1	M	28	intern	3/5/03	10/5/03	fever, cough, general malaise, rhinorrhoea, sore throat, headache	SARS ward	>640	+ve
2	F	21	nurse	8/5/03	8/5/03	fever, myalgia, headache	SARS ward	640	+ve
3	F	21	nurse	10/5/03	10/5/03	fever, chills, myalgia	SARS ward	160	+ve
4[a]	F	26	nurse	11/5/03	11/5/03	fever, cough, headache	SARS ward	640	+ve
5	F	23	nurse	13/5/03	13/5/03	fever, myalgia, headache	SARS ward	>640	+ve
6	M	29	resident	13/5/03	nil	general malaise	SARS ward	>640	+ve
7[a]	F	25	nurse	15/5/03	15/5/03	fever, chills, vomiting	SARS ward	160	+ve
8[a]	M	44	researcher	10/12/03	16/12/03	fever, cough, diarrhoea	laboratory	>640	+ve

[a]Infected HCW who received convalescent plasma.

Table 2. Variation of viral load, IgM and IgG before and after convalescent plasma transfusion

HCW	4	7	8
Viral load ($\times 10^3$ copies/mL)			
before transfusion	495	76	650
after transfusion[a]	0	0.001	0
IgM (units of fluorescent intensity)			
before transfusion	879	144	69
after transfusion			
day 1	730	1516	223
day 3	1872	7982	307
day 5	NA	5731	525
>1 month	84	104	NA
IgG (units of fluorescent intensity)			
before transfusion	3461	1124	254
after transfusion			
day 1	5191	3388	1594
day 3	9124	58252	2476
day 5	NA	56729	8259
>1 month	1012	1199	NA

NA, not available.
[a]Tested after 18–24 h.

Prognosis

Thoracic CT scans and pulmonary function tests with diffusion capacity were performed for HCW 4, 7 and 8. Pulmonary fibrosis and mildly restrictive ventilatory impairment were identified in HCW 4. Normal pulmonary function and diffusion capacity was observed post discharge for HCW 7 and 8. Subsequently HCW 7 became pregnant and she delivered a healthy baby 13 months after discharge. Anti-SARS-CoV IgG was detected in both mother and infant after delivery. Thus, we suspect passive transfer of anti-SARS-CoV antibody in the infant, rather than congenital infection of the infant.

Discussion

Since the SARS epidemic, no clinical treatment has been proved consistently effective against SARS-CoV infection.[9] Although a number of compounds with *in vitro* activity against CoV have been identified,[10] no clinical data on the use of such compounds have been obtained.

In two previous reports, convalescent plasma transfusions were found to be more effective when the patients were given this treatment before day 14 of the illness and seroconversion.[17,18] Our study further disclosed that virus was cleared 1 day after convalescent plasma was transfused, followed by subsidence of fever and resolution of pulmonary infiltrates. Anti-SARS IgM and IgG were subsequently documented after transfusion. No significant side effects were found.

Although a favourable outcome was achieved in our patients, the role of effectiveness of convalescent plasma therapy remains inconclusive. The sample size in this study was too small and other treatments including ribavirin, methylprednisolone, IVIG and protease inhibitors had also been used concomitantly, confounding the result. The efficacy of the convalescent plasma for SARS infection needs to be studied further. The survival of the two HCW and laboratory researcher suggest that a randomized trial of such therapy is ethically acceptable especially for individuals not responding to ribavirin or methylprednisone. When SARS patients develop severe progression and fail to respond to the available treatment, alternative therapy with convalescent plasma transfusion may be considered.

Transparency declarations

All authors declare no conflict of interests to any commercial and government policy.

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