

Detection of Severe Acute Respiratory Syndrome Coronavirus RNA in Plasma during the Course of Infection

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We examined severe acute respiratory syndrome-associated coronavirus (SARS-CoV) RNA in plasma of 32 patients (probable SARS cases) by a quantitative real-time reverse transcription-PCR assay and reported that the highest detection rate, 75%, was found between day 5 and day 7 of illness, followed by rates of 64, 50, and 38% found between day 8 and day 11, day 2 and day 4, and day 12 and day 16, respectively. Analysis of sequential SARS-CoV load in plasma from six cases revealed different patterns of viremia, with the peak between day 4 and day 8. Our findings of the high detection rate of SARS-CoV RNA in plasma before day 11, together with the relative convenience of collecting and handling plasma, suggest that plasma can be used for early diagnosis of SARS.

Severe acute respiratory syndrome (SARS) is an emerging infectious disease that poses a potential threat to the health of people throughout the world (10, 16). The etiological agent is a novel coronavirus (CoV), the SARS-associated CoV (SARS-CoV) (4, 6, 10). Because of the relative high transmissibility and mortality of the disease, early diagnosis and implementation of control measures are important for control of the disease (10). Several PCR-based molecular assays have been developed to detect and/or quantify SARS-CoV RNA in various respiratory specimens, including sputum, nasopharyngeal aspirates-swab, and throat swab-wash, for early detection of SARS-CoV (4–6, 11, 12, 14, 19). Collection and processing of respiratory specimens require compliance with the guidelines for aerosol-generating procedures and use of a biosafety level 2 (BSL-2) facility with stringent BSL-3 work practices (1, 2). In contrast, blood samples are easier and safer to collect and can be processed using BSL-2 work practices (1).

Detection of SARS-CoV RNA in plasma was first reported to be of low sensitivity (4). Subsequent studies involving more cases revealed that SARS-CoV RNA can be detected in plasma with a positive rate of 50% in the first 7 or 14 days and in serum with a positive rate of 78% for samples collected at admission (8, 9). Another study revealed sequential changes of SARS-CoV load in the plasma of eight pediatric patients and a higher detection rate, suggesting that plasma can be used for early diagnosis (7). However, the detection rates of SARS-

CoV in plasma at different time points of illness as well as sequential changes in SARS-CoV load in the plasma of adult patients remain largely unclear. In this study, we examined SARS-CoV RNA in plasma from patients of probable SARS cases during the course of infection by using a previously described quantitative real-time reverse transcription PCR (RT-PCR) assay (14). Moreover, we investigated sequential changes of SARS-CoV load in plasma from six patients. Our findings demonstrate the dynamic nature of plasma viral load and provide important information regarding the best timing for collecting plasma samples to detect SARS-CoV.

The study included 32 adult patients who met the World Health Organization clinical case definitions of probable SARS (17) and were admitted to the National Taiwan University Hospital in April and May 2003 during the SARS outbreak in Taipei. All patients have been confirmed as SARS patients by serological tests as described previously (5). The first day of fever is defined as day 1 of illness. After the diagnosis, all patients received oral ribavirin for 10 days unless adverse effects were noted as described previously (13). With the patients' consent, blood samples were collected in the EDTA-containing tubes, and plasma was prepared (1). As negative controls, blood samples were collected from nine healthy persons as well as from nine non-SARS patients who were admitted at the same period and excluded from the SARS patient group by serological testing 28 days later (5). Viral RNA was extracted from 280 μ l of plasma by use of a QIAamp viral RNA Mini kit (QIAGEN, Hilden, Germany). A previously described construct, ORF1b/pCRII-TOPO, which contained a highly conserved region of ORF1b of SARS-CoV, was used to generate the in vitro-transcribed RNA by SP6 transcription (Promega, Madison, Wis.) (14). An aliquot (5 μ l) of RNA isolated from plasma and known amounts of the in vitro-transcribed RNA (5 to 50,000,000 copies) were subjected to a real-time RT-PCR assay, which utilized a TaqMan one-step

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TABLE 1. Detection rate and SARS-CoV load in plasma during the course of infection in SARS patients (probable SARS cases)^a

Day of illness ^b	No. of samples	No. of positive samples	Detection rate (%)	Viral load (copies/ml of plasma) ^c		
				Median	Mean	Range
d2–d4	20	10	50	2.36×10^3	4.17×10^3	2.15×10^2 – 5.89×10^5
d5–d7	12	9	75	6.91×10^3	1.41×10^4	1.80×10^2 – 3.44×10^6
d8–d11	11	7	64	1.47×10^4	2.34×10^4	2.91×10^3 – 1.20×10^5
d12–d16	8	3	38	3.83×10^3	2.82×10^3	4.01×10^2 – 1.48×10^4
Total	51	29	57			

^a Probable SARS cases were diagnosed according to the WHO clinical definitions (17).

^b The first day of fever is the day 1 (d1) of illness.

^c The medians, means, and ranges of viral load of the positive samples are shown.

RT-PCR master mix reagent kit (Applied Biosystems, Foster City, Calif.) and SAR1S_AS TaqMan primers and probe within a region of ORF1b as described previously (14). The linear range of the assay reaches 8 logs. The sensitivity of the assay is five copies of RNA per reaction, corresponding to 178 copies per ml of plasma.

Of the 32 patients, 11 were male and 21 were female. The ages of these patients ranged from 21 to 75 years (mean, 41.2). Of the 51 plasma samples collected between day 2 and day 16 from these patients, SARS-CoV RNA was detected in 29, corresponding to an overall detection rate of 57% (Table 1). None of the plasma samples from the 9 healthy controls and 9 non-SARS patients had detectable SARS-CoV RNA (data not shown). The viral load in the positive-testing samples ranged from 2.15×10^2 to 3.44×10^6 copies/ml; these results were in the same range as those reported previously (7–9). To analyze the detection rate at different time points after the onset of illness, the samples were further grouped according to the day of illness. The highest detection rate was 75%, which was found between day 5 and day 7. The detection rates were 64, 50, and 38% for samples collected between day 8 and day 11, day 2 and day 4, and day 12 and day 16, respectively. The viral load in the group between day 5 and day 7, which had the highest detection rate, was not significantly higher than those in other three groups, including the groups between day 2 and day 4, day 8 and day 11, and day 12 and day 16 ($P = 0.083$, 0.925 , and 0.087 , respectively) (Mann-Whitney test).

To further investigate the changes of SARS-CoV load in plasma during the course of infection, sequential plasma samples from 6 of the 32 patients were examined. As shown in Fig. 1, the peak of plasma viral load was noted between day 4 and day 8 (median, day 6.5). While some patients had a pattern of transient viremia (Fig. 1A and B), others had viremia for more than 10 days; SARS-CoV remained detectable until day 19 to day 21 in three cases (Fig. 1D to F). For patients ID14 and ID2, SARS-CoV RNAs were undetectable in the follow-up sera after day 30 (data not shown). This finding indicates that the patterns of viremia differ among different patients and that some patients have a more protracted viremia. When the ribavirin usage characteristics were compared, a drop in viral load was found for three patients (Fig. 1A to C). However, viral RNA remained detectable despite more than 10 days of ribavirin therapy for two patients (Fig. 1D and F). This finding is consistent with our previous observation of the effect of ribavirin on viral

load of throat wash (15) and suggests that ribavirin is not effective in reducing viral load in some patients.

To our knowledge, this is the first study reporting the pattern of viremia of SARS-CoV for adult patients during the course of infection. Two patients had a pattern of transient viremia, and four patients had viremia of longer duration. This is in agreement with the patterns reported for eight pediatric SARS patients: transient viremia was found for two patients and a protracted pattern was found for six (7). Of the four patients who had a protracted pattern of viremia, three had SARS-CoV RNA detectable between day 19 and day 21 (Fig. 1D to F). This is consistent with a previous report that SARS-CoV RNA can be detected in serum up to day 23 to day 29 and suggests that blood samples are potentially contagious even after day 20 (5). Another patient, ID1, required intubation and a ventilator at day 10 (Fig. 1C). Whether patients with viremia of longer duration have a more severe and complicated disease course needs to be investigated with more cases in the future.

Present guidelines of samples collection for detection of SARS-CoV recommend three types of specimens from the upper respiratory tract, including nasopharyngeal aspirates, nasopharyngeal swabs, and oropharyngeal swabs (3, 18). However, the RT-PCR-positive rates of nasopharyngeal aspirates in a study were 32% at day 3, 50% at day 5, and 68% at day 14 of illness (11). Another study reported a positive rate of 71% with a mean at day 4.4 (12). Similarly, the RT-PCR-positive rate of throat swab reported in another study was 32% at day 2 of illness, reached 50 to 60% between day 7 and day 10, and declined thereafter (19). We studied plasma samples of SARS patients and report here that the highest detection rate of 75% was found between day 5 and day 7, followed by 64% between day 8 and day 11, 50% between day 2 and day 4, and 38% between day 12 and day 16. Consistent with this trend, analysis of sequential samples revealed that the peak plasma viral load was found between day 4 and day 8. Compared with respiratory specimens, plasma samples are easier and more convenient to collect and handle. These features, together with the high detection rate of SARS-CoV in plasma before day 11, suggest that plasma can be used for early diagnosis of SARS.

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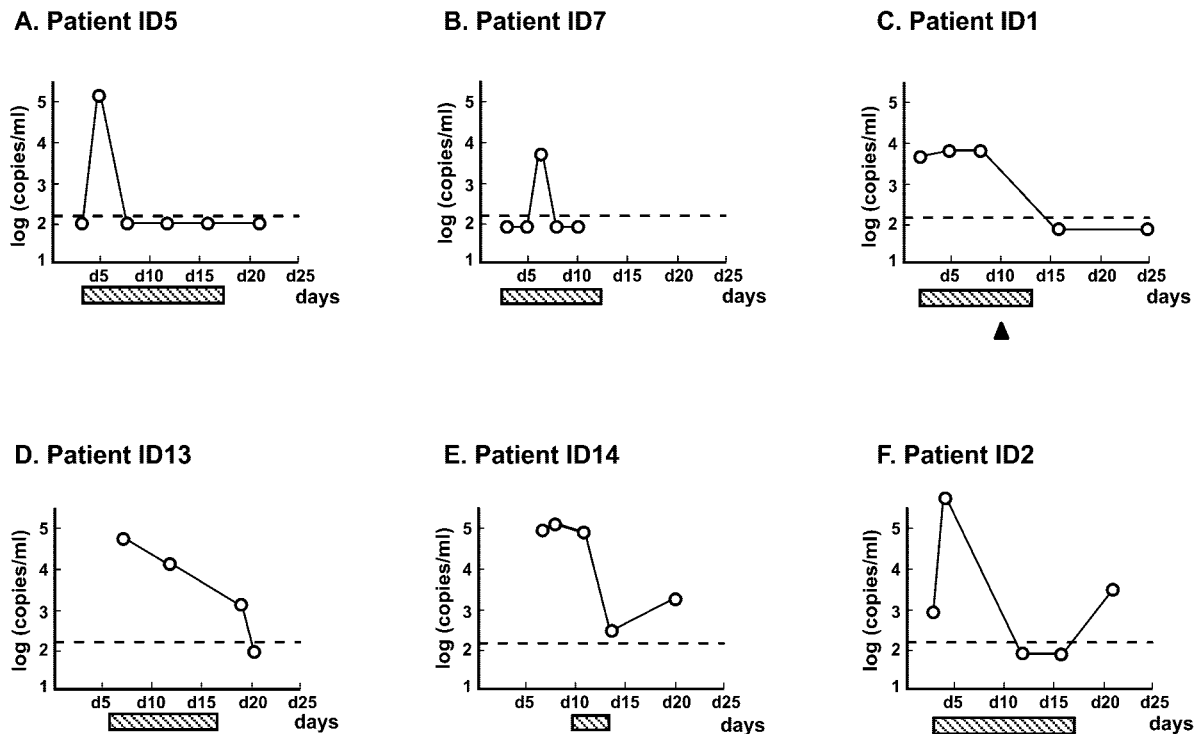


FIG. 1. Sequential changes of SARS-CoV load in plasma during the course of infection in six patients suspected of having SARS (probable SARS cases) (A to F) (17). SARS-CoV loads in plasma were determined by a real-time RT-PCR assay (14). Day 1 (d1) is the first day of fever. Dashed lines indicate the limit of detection (178 copies/ml of plasma). Hatched bars indicate ribavirin therapy; the closed triangle indicates intubation (in patient ID1).

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