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# Viral Replication in the Nasopharynx Is Associated with Diarrhea in Patients with Severe Acute Respiratory Syndrome

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The role of severe acute respiratory syndrome (SARS) coronavirus as an enteric pathogen was investigated in a cohort of 142 patients with SARS who were treated with a standard treatment protocol. Data from daily hematological, biochemical, radiological, and microbiological investigations were prospectively collected, and the correlation of these findings with diarrhea was retrospectively analyzed. Sixty-nine patients (48.6%) developed diarrhea at a mean ( $\pm$  standard deviation [SD]) of  $7.6 \pm 2.6$  days after the onset of symptoms. The diarrhea was most severe at a mean ( $\pm$  SD) of  $8.8 \pm 2.4$  days after onset, with a maximum frequency of 24 episodes per day (median, 5 episodes; range, 3–24 episodes). A higher mean virus load in nasopharyngeal specimens obtained on day 10 after the onset of symptoms was significantly associated with the occurrence of diarrhea ( $3.1 \log_{10}$  vs.  $1.8 \log_{10}$  copies/mL;  $P = .01$ ) and mortality ( $6.2$  vs.  $1.7 \log_{10}$  copies/mL;  $P < .01$ ). However, diarrhea was not associated with mortality. The lung and the gastrointestinal tract may react differently to SARS coronavirus infection. Additional investigation of the role of SARS coronavirus in the pathogenesis of diarrhea in patients with SARS should be conducted.

The severe acute respiratory syndrome (SARS) pandemic has affected >8000 patients, with 774 fatalities [1]. SARS is caused by a novel coronavirus, which was consistently isolated from patients with SARS who had subsequent seroconversion [2–4]. Similar histopathological findings of SARS and seroconversion have been reproduced in cynomolgus macaques (*Macaca fascicularis*) that were artificially inoculated with the same SARS coronavirus [5]. Subsequent detailed histopathological study of these infected macaques confirmed that the inflammatory changes were confined to the

lungs [6]. Thus, SARS is largely regarded as a novel viral pneumonia. However, in a prospective clinical study of a cohort of patients with SARS, diarrhea was noted in 1% of patients at hospital admission and in 73% during hospitalization [7]. Viral genomes or the virus could be detected by RT-PCR or cell culture of stool samples obtained from these patients [8]. As illustrated by the example of enterovirus infection, shedding of virus in stool does not necessarily imply the presence of disease in the intestinal tract. Nevertheless, presence of numerous coronavirus particles was demonstrated in the terminal ileum and colon in a patient with SARS who had diarrhea [9]. It would be interesting to know whether the SARS coronavirus indeed causes diarrhea, because histopathological examination of intestinal biopsy specimens does not reveal any inflammatory or cytolytic damages.

We have previously shown that specimens obtained from the nasopharynx are useful for the diagnosis of

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rotavirus infection in children [10]. This is not unexpected, because an earlier study also demonstrated the presence of rotavirus in nasopharyngeal secretions from children with upper respiratory tract infection [11]. In this retrospective study, we attempt to correlate the virus load of SARS coronavirus shedding from the nasopharynx, the upper end of the aerodigestive tract, with the presence of diarrhea in a cohort of patients with SARS. Demographic characteristics, clinical features, and hematological and radiological findings of patients with SARS who had or did not have diarrhea were collected and analyzed.

## PATIENTS, MATERIALS, AND METHODS

The clinical records for all 142 patients whose cases fulfilled the modified World Health Organization definition of SARS [4, 7] and who were treated at the United Christian Hospital and Caritas Medical Centre (Hong Kong) were analyzed. The daily clinical findings from history and physical examinations and hematological, biochemical, radiological, and microbiological investigations were prospectively collected and analyzed. In brief, the case definition includes fever (temperature,  $\geq 38^{\circ}\text{C}$ ), cough or shortness of breath, and new pulmonary infiltrates noted on chest radiographs or high-resolution CT, in the absence of an alternative diagnosis to explain the clinical presentation. Some of the clinical and virological results for the first 75 patients were previously reported [7]. All patients were

treated with amoxicillin-clavulanate (1.2 g q8h iv) and azithromycin (500 mg q.d. po). In patients with a known allergy to penicillin, we administered 500 mg of oral levofloxacin every 24 h. As soon as the diagnosis of SARS was established, ribavirin (a 4-g oral loading dose, followed by 1.2 g q8h or 8 mg/kg q8h iv if the patient could not tolerate oral treatment) was given for 14 days, in addition to a tailing regimen of hydrocortisone (starting dose, 200 mg q8h iv) for 10 days, followed by oral prednisolone (1 mg/kg for 5 days, 0.5 mg/kg for 3 days, and 0.25 mg/kg for 3 days) for 11 days. Pulses of methylprednisolone (500–1000 mg q.d. iv for up to 3 g) were administered to patients with clinical deterioration. All hepatitis B surface antigen-positive patients were given prophylactic lamivudine (100 mg q.d. po) while taking corticosteroids.

Patients were prospectively monitored for development of diarrhea during hospitalization. Diarrhea was defined as  $\geq 3$  bowel movements per day for  $\geq 2$  consecutive days. The occurrence of diarrhea in relation to the onset of symptoms of SARS, the frequency of bowel movements, and the duration of diarrhea were recorded. Investigation for other causes of diarrhea was performed, including culture for *Clostridium difficile*, cell culture assay for detection *C. difficile* cytotoxin, and ELISA (IDEIA Rotavirus; DAKO) for detection of rotavirus.

For the diagnosis of coronavirus infection, nasopharyngeal specimens and serum samples were obtained at hospital admission. The convalescent-phase serum sample was taken 14–28 days after the onset of symptoms. For all patients, qualitative and quantitative RT-PCRs for SARS coronavirus were retrospectively performed using the nasopharyngeal specimens obtained at admission and 10 days after the onset of symptoms. Stool and urine specimens were obtained for RT-PCR during the hospital stay. All virological diagnostic protocols, including performance of qualitative and quantitative RT-PCRs, rapid viral antigen detection tests, viral cultures, immunofluorescent antibody tests for detection for IgG seroconversion against SARS-associated coronavirus, and other microbiological diagnostic evaluations, were done in the manner described in our previous publications [4, 7].

**Statistical analysis.** All data were calculated from the day of onset of clinical symptoms. We compared the demographic characteristics and laboratory values for patients with and without diarrhea by means of Fisher's exact test for categorical variables and Student's *t* test or the Mann-Whitney *U* test for continuous variables. A 2-tailed *P* value of  $<.05$  was considered to be statistically significant. We used SPSS software, version 11.0 (SPSS), for all analyses.

## RESULTS

Of the 142 patients recruited in this study, 138 (97.2%) were ethnic Chinese, and the remaining patients were Filipinos. The

**Table 1. Clinical symptoms and signs for 142 patients with severe accurate respiratory syndrome at presentation.**

Clinical feature	No. (%) of patients
Symptom	
Fever	137 (96.5)
Chill	96 (67.6)
Rigors	60 (42.3)
Myalgia	82 (57.7)
Malaise	27 (19.0)
Headache	22 (15.5)
Sore throat	13 (9.2)
Cough	41 (28.9)
Shortness of breath	4 (2.8)
Diarrhea	15 (10.6)
Sign	
Oxygen desaturation <sup>a</sup>	2 (1.4)
Tachypnea <sup>b</sup>	11 (7.7)
Tachycardia <sup>c</sup>	48 (33.8)

<sup>a</sup> Oxygen saturation, oximetry,  $<90\%$ .

<sup>b</sup> Respiratory rate,  $>20$  breaths/min.

<sup>c</sup> Heart rate,  $>100$  beats/min.

**Table 2. Hematological and biochemical findings for 142 patients with severe acute respiratory syndrome at presentation and on day 10 after the onset of symptoms.**

Variable	At presentation	On day 10 after onset of symptoms	Normal range
Hemoglobin level			11.5–16.5 g/dL
Mean g/dL $\pm$ SD	13.4 $\pm$ 0.15	13.1 $\pm$ 0.79	
No. (%) of patients with anemia	13 (9.2)	23 (16.2)	
Hematocrit			0.35–0.45
Mean $\pm$ SD	0.40 $\pm$ 0.44	0.37 $\pm$ 0.43	
No. (%) of patients	...	...	
Total WBC count			4–11 $\times 10^9$ cells/L
Mean $\times 10^9$ cells/L $\pm$ SD	6.2 $\pm$ 2.3	13.9 $\pm$ 4.4	
No. (%) of patients with leukocytosis	4 (2.8)	88 (62.0)	
Neutrophil count			2.0–7.5 $\times 10^9$ neutrophils/L
Mean $\times 10^9$ neutrophils/L $\pm$ SD	5.2 $\pm$ 2.0	12.3 $\pm$ 4.4	
No. (%) of patients with neutrophilia	21 (14.8)	118 (83.1)	
Lymphocyte count			1.5–4.0 $\times 10^9$ lymphocytes/L
Mean $\times 10^9$ lymphocytes/L $\pm$ SD	0.92 $\pm$ 0.50	0.57 $\pm$ 0.26	
No (%) of patients with severe lymphopenia <sup>a</sup>	82 (57.7)	109 (76.8)	
Platelet count			150–400 $\times 10^9$ platelets/L
Mean $\times 10^9$ platelets/L $\pm$ SD	173 $\pm$ 50	233 $\pm$ 64	
No. (%) of patients with thrombocytopenia	44 (31.0)	8 (5.6)	
Sodium level			136–148 mmol/L
Mean mmol/L $\pm$ SD	137 $\pm$ 4	143 $\pm$ 3	
No. (%) of patients with hyponatremia	27 (19.0)	3 (2.1)	
Potassium level			3.4–3.6 mmol/L
Mean mmol/L $\pm$ SD	3.9 $\pm$ 0.4	3.4 $\pm$ 0.5	
No. (%) of patients with hypokalemia	15 (10.6)	65 (45.8)	
Urea level			2.5–6.4 mmol/L
Mean mmol/L $\pm$ SD	4.1 $\pm$ 1.3	5.3 $\pm$ 1.8	
No. (%) of patients with elevated urea level	5 (3.5)	24 (16.9)	
Creatinine level			60–106 $\mu$ mol/L
Mean $\mu$ mol/L $\pm$ SD	86 $\pm$ 16	72 $\pm$ 12	
No. (%) of patients with elevated creatinine level	14 (9.9)	1 (0.7)	
ALT level			5–41 IU/L
Mean IU/L $\pm$ SD	31 $\pm$ 34	55 $\pm$ 47	
No. (%) of patients with elevated ALT level	27 (19.0)	59 (41.5)	
AST level			12–38 IU/L
Mean IU/L $\pm$ SD	36 $\pm$ 30	25 $\pm$ 16	
No. (%) of patients with elevated AST level	30 (21.1)	20 (14.1)	
Albumin level			42–54 g/L
Mean g/L $\pm$ SD	40 $\pm$ 4	32 $\pm$ 3	
No. (%) of patients with low albumin level	77 (54.2)	142 (100)	
CPK level			38–174 IU/L
Mean IU/L $\pm$ SD	173 $\pm$ 213	74 $\pm$ 75	
No. (%) of patients with elevated CPK level	36 (25.4)	7 (4.9)	
LDH level			200–360 IU/L
Mean IU/L $\pm$ SD	376 $\pm$ 151	489 $\pm$ 204	
No. (%) of patients with elevated LDH level	39 (27.5)	66 (46.5)	

**NOTE.** ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatinine kinase; LDH, lactate dehydrogenase.

<sup>a</sup> Lymphocyte count,  $<1 \times 10^9$  lymphocytes/L.

**Table 3. Extent of radiological involvement and microbiological findings for 142 patients with severe acute respiratory syndrome (SARS).**

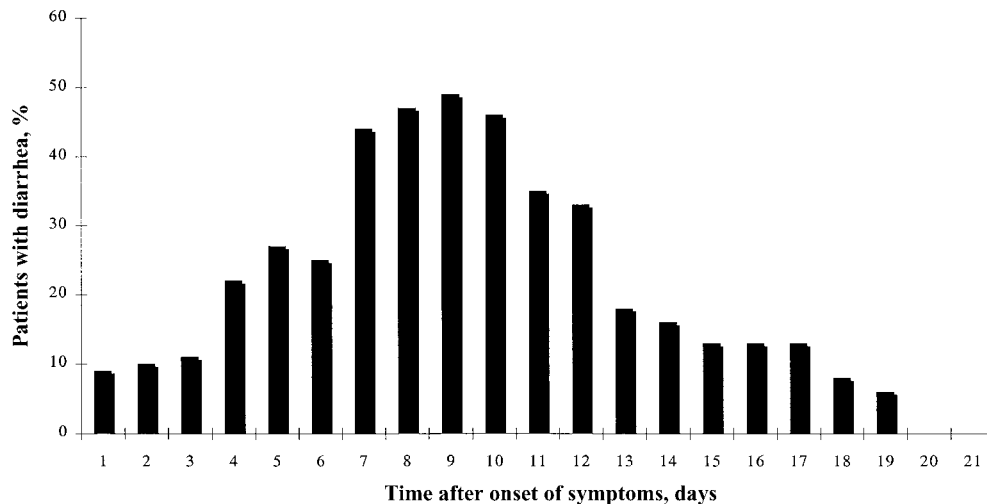
Radiological or microbiological finding	No. (%) of patients
Radiological examination	
Abnormal chest radiograph finding	108 (76.1)
Multizone involvement	29 (20.4)
Single-zone involvement	79 (55.6)
Right lower zone lesion	27
Right middle zone lesion	13
Right upper zone lesion	6
Left lower zone lesion	15
Left middle zone lesion	9
Left upper zone lesion	9
Abnormal finding of thorax CT <sup>a</sup>	34 (23.9)
Multi-zone involvement	15 (10.6)
Single-zone involvement	19 (13.4)
Right lower zone lesion	1
Right middle zone lesion	1
Right upper zone lesion	4
Left lower zone lesion	12
Left middle zone lesion	1
Left upper zone lesion	0
Subpleural lesions	8 (5.6)
Predominant radiological lesions	
Consolidation	16 (11.3)
Ground-glass appearance	6 (4.2)
Combination of consolidation and ground-glass appearance	12 (8.5)
Microbiological examination	
Four-fold increase in antibody against SARS coronavirus	130 (91.5)
Positive qualitative RT-PCR result for any clinical specimen	109 (76.8)
Nasopharyngeal specimen obtained at hospital admission	45 (31.7)
Nasopharyngeal specimen obtained during hospitalization	71 (50.0)
Stool specimen obtained during hospitalization	101 (71.1)
Urine specimen obtained during hospitalization	39 (27.5)
Either serological or RT-PCR-based confirmation of SARS coronavirus	137 (96.5)

<sup>a</sup> Performed for 34 patients with apparently normal chest radiograph findings.

patients were admitted to the hospital  $2.4 \pm 1.8$  days after onset of symptoms. There were 56 male and 86 female patients (ratio, 0.65:1), and the mean age was  $40.4 \pm 13.9$  years (range, 20–86 years). Eighty-six patients (60.6%) were household contacts of patients with SARS, and 28 (19.7%) were health care workers. For 28 patients (19.7%), the source of SARS infection could not be traced. Underlying chronic disease was found in 21 patients (14.8%), of whom 12 were chronic hepatitis B carriers without stigmata of chronic liver disease, 3 had diabetes mellitus, 3 had ischemic heart disease, 2 had malignancies, and 1 had chronic obstructive airway disease.

The clinical symptoms and signs of these 142 patients are shown in table 1. Common presenting symptoms included sys-

temic upset, such as fever (96.5% of patients), chills (67.6%), and myalgia (57.7%). One-third of patients had tachycardia noted at admission. With regard to hematological and biochemical manifestations of disease, severe lymphopenia (lymphocyte count,  $<1 \times 10^9$  lymphocytes/L) was present in 82 patients (57.7%), and thrombocytopenia was present in 44 patients (31.0%) (table 2). Hyponatremia was noted in 27 patients (19%). Elevations in alanine aminotransferase, aspartate aminotransferase, creatinine kinase, and lactate dehydrogenase levels were noted in 27 (19.0%), 30 (21.1%), 36 (25.4%), and 39 (27.5%) of the patients, respectively. Initial chest radiograph findings were abnormal for 108 patients (76.1%), and multi-zone involvement was found in 29 of them (table 3). In 79



**Figure 1.** Percentage of patients with severe acute respiratory syndrome who had diarrhea during hospitalization

patients who presented with single-zone lesions, the right lower and left lower zones were more commonly involved than were the other zones. Thoracic CT was performed for 34 patients with initial apparently normal chest radiograph findings. The lesions were mostly confined to the retrocardiac region in the left lower lobes.

With regard to the microbiological evaluation for SARS coronavirus, 137 patients (96.5%) had either a 4-fold increase in antibody to SARS coronavirus or positive results of RT-PCR of nasopharyngeal, stool, or urine specimens (table 3). Four-fold increases in antibody titers to SARS coronavirus were found in 26 of 28 patients who did not have any history of epidemiological exposure, and RT-PCR results were positive for the remaining 2 patients.

During the course of infection, diarrhea occurred in 69 patients (48.6%). The distribution of episodes of diarrhea during the first 3 weeks of hospitalization is shown in figure 1. Diarrhea occurred  $7.6 \pm 2.6$  days after the onset of symptoms and became most severe on day  $8.8 \pm 2.4$ , with a maximum frequency of 24 episodes per day (median, 5 episodes per day; range, 3–24 episodes per day). The diarrhea lasted for a duration of  $3.8 \pm 2.3$  days in this cohort, resulting in prerenal azotemia in 6 patients and electrolyte disturbances in 39 patients. The diarrhea was rather painless, with no blood or mucus present in the stool for any patients. None of the patients had positive results of culture for *C. difficile* or cell culture assay for *C. difficile* cytotoxin. All stool samples were negative for rotavirus on ELISA tests.

The clinical characteristics of patients with and those without diarrhea are summarized in table 4. There were no differences between the 2 groups with regard to age, sex, comorbidities, and chronic hepatitis B status. The manifestations on chest radiographs at hospital admission were also similar. There were

no statistical differences in hematological and biochemical parameters between the 2 groups at presentation and on day 10 after the onset of symptoms. Patients with diarrhea had lower potassium levels ( $3.3 \pm 0.5$  mmol/L) and higher mean sodium levels ( $143 \pm 3$  mmol/L). There was no difference in the mean virus loads in nasopharyngeal specimens (as measured by quantitative RT-PCR) at baseline between the groups with and without diarrhea. A higher mean virus load in nasopharyngeal specimens obtained on day 10 after the onset of symptoms was significantly associated with the occurrence of diarrhea ( $3.1$  vs.  $1.8$  log<sub>10</sub> copies/mL;  $P = .01$ ) and mortality ( $6.2$  vs.  $1.7$  log<sub>10</sub> copies/mL;  $P < .01$ ). However, there were no differences between patients with and those without diarrhea with regard to the incidence of intensive care admission, receipt of mechanical ventilatory support, length of hospital stay, and overall mortality. Furthermore, the severity of diarrhea had no correlation with the virus load in nasopharyngeal specimens and overall mortality (figure 2).

## DISCUSSION

SARS is predominantly a viral pneumonia with a rapid tempo of deterioration. However, extrapulmonary manifestations are not uncommon. Skin rash [4], petechial cutaneous bleeding with prolonged activated partial thromboplastin time and increased d-dimer [12], impaired liver function, subclinical left ventricular diastolic dysfunction [13], and rhabdomyolysis [14] were occasionally reported. One of the more common presentations other than respiratory symptoms is diarrhea [4, 7, 9, 15–18]. Our previous report that >70% of patients in the Amoy Garden cohort developed diarrhea showed that fecal excretion may be an important mode of shedding and transmission [7]. Subsequent epidemiological investigation confirmed the im-

**Table 4. Demographic characteristics of 142 patients who had severe acute respiratory syndrome with or without diarrhea during hospitalization.**

Characteristic	Diarrhea during hospitalization		P
	Present (n = 69)	Absent (n = 73)	
Age, mean years ± SD	38.9 ± 13.1	41.7 ± 14.6	.24
Sex			
Male	25	31	.45
Female	44	42	
Comorbidities, including chronic hepatitis B	11 (15.9)	10 (13.7)	.87
Chronic hepatitis B	8 (11.6)	4 (5.5)	.19
Duration of symptoms before hospital admission, mean days ± SD	2.4 ± 2.8	2.1 ± 2.9	.59
Apparently abnormal chest radiograph finding at hospital admission	54 (78.3)	54 (74.0)	.55
Multilobar involvement on initial chest radiograph	15 (21.7)	14 (19.2)	.71
Virus load in nasopharyngeal specimens, <sup>a</sup> mean log <sub>10</sub> copies/mL ± SD			
At hospital admission	2.3 ± 2.8	2.1 ± 2.9	.69
At day 10 after onset of symptoms	3.1 ± 3.2	1.8 ± 2.9	.01
Laboratory values at day 10 after onset of symptoms, mean ± SD			
Hematocrit	0.37 ± 0.04	0.37 ± 0.04	1.00
Lymphocyte count, ×10 <sup>9</sup> lymphocytes/L	0.61 ± 0.29	0.57 ± 0.36	.46
Sodium level, mmol/L	143 ± 3	142 ± 3	.02
Potassium level, mmol/L	3.3 ± 0.5	3.5 ± 0.5	<.01
Urea level, mmol/L	5.2 ± 1.7	5.4 ± 1.7	.47
Creatinine level, μmol/L	71 ± 11	72 ± 12	.81
Alanine aminotransferase level, IU/L	60 ± 49	47 ± 40	.10
Albumin level, g/L	32 ± 3	32 ± 4	.66
Lactate dehydrogenase level, IU/L	490 ± 193	480 ± 215	.82
Length of hospital stay, mean days ± SD	33 ± 11	31 ± 10	.37
Intensive care admission	19 (27.5)	16 (21.9)	.44
Receipt of mechanical ventilatory support	16 (23.2)	10 (13.7)	.14
Overall mortality	14 (20.3)	11 (15.1)	.41

**NOTE.** Data are no. (%) of patients, unless otherwise indicated.

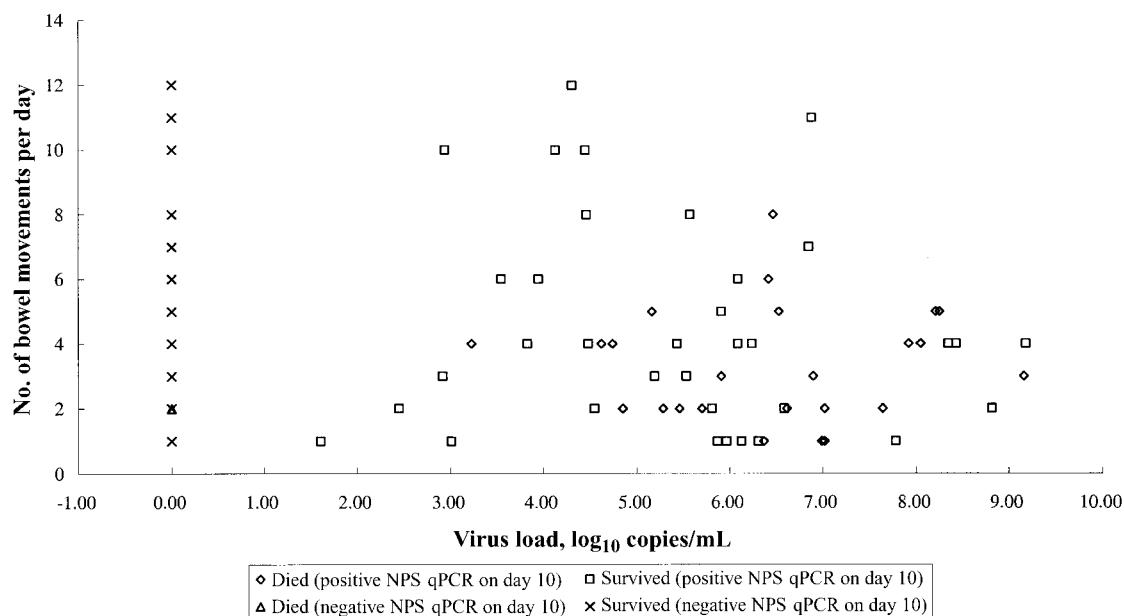
<sup>a</sup> Determined by quantitative RT-PCR.

portance of faulty sewage systems in propagating this massive outbreak of disease. Retrospective virological examination of stool samples by RT-PCR confirmed the presence of the virus in >90% of the samples obtained between day 14 and 21 after onset of symptoms, which is almost twice the positivity rate of the corresponding nasopharyngeal samples [8]. It is therefore interesting to examine the role of SARS coronavirus in the pathogenesis of diarrhea in patients with SARS.

Diarrhea is not an uncommon symptom during hospitalization for patients with infectious diseases other than acute gastroenteritis. In fact, diarrhea has been well reported in pneumonic illnesses other than SARS, such as legionnaires disease [19], *Pneumocystis carinii* pneumonia [20], and influenza (especially in children) [21]. Moreover, diarrhea is not uncommon in association with many systemic infections. For example, the rate of diarrhea was reported to be 34.5% among children with

primary dengue fever [22]. The exact mechanisms leading to diarrhea in these systemic or pulmonary infections were not known, but they could be related to the cytokine profiles or metabolic changes associated with sepsis. In addition to primary viral illness, other potential causes of diarrhea include the use of drugs during hospitalization and *C. difficile* superinfection, because most patients with SARS have been empirically treated with antibiotics that cover typical and atypical pneumonia. Finally, diarrhea can be the direct result of viral cytolysis, because the virus can grow and produce a cytopathic effect in a large intestinal cell line, CACO-2 [23]. A study of the correlation between the clinical parameters, microbiological findings, and outcomes for patients with SARS with and without diarrhea may differentiate the relative importance of the suggested causative factors.

In this cohort of 142 patients with SARS, only 10.6% of the



**Figure 2.** Frequency of bowel movements in relation to virus load in nasopharyngeal specimens on day 10 after the onset of symptoms. NPS, nasopharyngeal specimen; qPCR, quantitative RT-PCR.

patients had diarrhea at hospital admission. During hospitalization, 69 (48.6%) of the patients had developed diarrhea at a mean time of  $7.6 \pm 2.6$  days after the onset of symptoms. The most severe diarrhea occurred 1–2 days after the onset of diarrhea, with a maximum frequency of 24 episodes per day. In 6 patients, diarrhea was so severe that intravenous fluid hydration was warranted. The diarrhea was relatively painless and watery, as we have reported previously [7]. No correlation was found between diarrhea and hematological changes, radiological changes, use of antibacterials, or isolation of *C. difficile* and rotavirus from stool specimens. The only significant correlation was that there was a higher virus load in the nasopharyngeal specimens taken from patients with diarrhea on day 10 after the onset of symptoms. The finding that a higher virus load—but not the presence of diarrhea—predicted mortality in this group of patients is not unexpected. In 2 previous studies of animal models of SARS [6, 24], histological changes of acute respiratory distress syndrome (ARDS) and inflammation—characterized by diffuse alveolar damage (disruption of alveolar walls and infiltration with neutrophils and macrophages), alveolar hyaline membrane formation, as well as multinucleated syncytial cells—were found in a macaque monkey model artificially inoculated with SARS coronavirus [6]. None of the monkeys developed diarrhea over a period of 16 days, despite the fact that RT-PCR results were positive for SARS coronavirus in 1 of the stool samples. Similarly, no histopathological changes could be detected in the intestines of the monkeys, despite there being concomitant florid pathological changes in the lungs [6]. These findings suggest that viral rep-

lication triggers a different response and thus different functional impairment in the lungs and the gastrointestinal tract. A high virus load in the nasopharynx specimens either may predict severe pneumonia, ARDS, and mortality in some patients, or it may predict just diarrhea in other patients. This postulation is in keeping with the lack of significant difference in hematological markers of inflammation or increased cell turnover (lactate dehydrogenase level) between the groups with or without diarrhea. Although suppression of inflammatory changes in the intestine resulting from steroid therapy is possible, the routine use of steroids and ribavirin as part of the treatment protocol did not allow us to control the effect of use of steroids or antivirals on diarrhea. Moreover, the use of steroids has not affected the severe inflammatory reactions in the lungs of persons who have died of SARS [25].

In our previous study, we showed that rotavirus can be detected by viral isolation in cell culture and by indirect immunofluorescent antigen detection [10]. This is not unexpected, because many viruses that affect the gastrointestinal tract also affect the respiratory tract, and vice versa. For instance, in several studies, rotavirus has been well reported to be associated with bronchiolitis and fatal pneumonitis in immunocompromised as well as immunocompetent hosts [11, 26, 27]. Others reported that respiratory symptoms were found in 93% of patients with gastroenteritis caused by enteric adenovirus [28]. This virus has been found in samples from the upper respiratory tract and in stool samples obtained from these patients [29]. Similar to rotavirus and adenovirus, the SARS coronavirus can multiply and shed in the mucosa of the upper



aerodigestive tract. In this regard, determination of the virus load in specimens from the nasopharynx may be useful for predicting the severity of illness in both the respiratory and the gastrointestinal tracts. From the laboratory perspective, RT-PCR is more likely to be affected by the abundant inhibitors present in stool than by nasopharyngeal secretions. Moreover, the volume of diarrhea varies markedly from patient to patient. This may also affect the interpretation of data on the virus load in stool. Therefore, we attempted to use nasopharyngeal specimens instead of stool specimens in this study.

Coronaviruses can cause respiratory or diarrheal diseases in both human and animals [30–44]. Human enteric coronavirus has been seen in diarrheal stool samples, but culture methods and characterization are still problematic [30, 31]. Human coronavirus OC43 and 229E are known to cause one-third of cases of the common cold [45, 46]. In the case of porcine coronavirus, a collection of transmissible gastroenteritis coronavirus (TGEV) recombinants were generated to study the molecular basis of TGEV tropism [47]. Recombinants of group 1 had enteric and respiratory tropism, whereas group 2 recombinants infected the respiratory but not the enteric tract. A substitution in amino acid 219 of the S protein in group 1 recombinant was responsible for the loss of enteric tropism [47]. On the other hand, targeted recombination of the S gene of TGEV resulted in a change from respiratory to enteric tropism and enhanced virulence [48]. However, no major deletion or mutation of functional significance had been identified by genomic sequencing of SARS coronavirus isolated from the diarrheal patients [49].

We have clearly demonstrated the presence of a numerous virus in tissue biopsy specimens from the terminal ileum and colon of a SARS patient with diarrhea [9]. The virus is capable of active replication inside the endoplasmic reticulum and adheres to the surface of enterocytes in both small and large intestines (as shown by electron microscopy), without causing significant villous atrophy or inflammatory cell infiltration in the mucosal or submucosal regions [9]. The absence of inflammation, cell necrosis, or microvilli atrophy does not exclude SARS coronavirus as the cause of diarrhea in these patients. For astrovirus-associated diarrhea in turkeys, the animal model has demonstrated mild histological changes, with a surprising lack of inflammation due to increased activation of the potent immunosuppressive cytokine transforming growth factor  $\beta$  at the peak of diarrhea during astrovirus infection [50]. Additional studies should be performed to ascertain the mechanism used by SARS coronavirus in the pathogenesis of diarrhea in humans.

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