

Fatal Severe Acute Respiratory Syndrome Is Associated with Multiorgan Involvement by Coronavirus

Gabriella A. Farcas,^{1,2,4} Susan M. Poutanen,^{3,5,7} Tony Mazzulli,^{2,3,5,7} Barbara M. Willey,^{5,7} Jagdish Butany,^{6,7} Sylvia L. Asa,^{6,7} Peter Faure,⁶ Poolak Akhavan,^{5,7} Donald E. Low,^{2,3,5,7} and Kevin C. Kain^{1,2,3,4,7}

¹McLaughlin-Rotman Center for Global Health, McLaughlin Center for Molecular Medicine, ²Faculty of Medicine, ³Department of Laboratory Medicine and Pathobiology, and ⁴Institute of Medical Science, University of Toronto, ⁵Department of Microbiology, Mount Sinai Hospital, ⁶Department of Pathology, University Health Network, and ⁷Toronto Medical Laboratories, Toronto, Canada

Severe acute respiratory syndrome (SARS) is characterized by pulmonary compromise; however, patients often have evidence of other organ dysfunction that may reflect extrapulmonary dissemination of SARS coronavirus (SARS-CoV). We report on the distribution and viral load of SARS-CoV in multiple organ samples from patients who died of SARS during the Toronto outbreak. SARS-CoV was detected in lung (100%), bowel (73%), liver (41%), and kidney (38%) in 19 patients who died of SARS, with the highest viral loads observed in lung (1.0×10^{10} copies/g) and bowel (2.7×10^9 copies/g). Fatal SARS was associated with multiorgan viral dissemination in a distribution that has implications for disease manifestation, viral shedding, and transmission.

An atypical and severe pneumonia (severe acute respiratory syndrome [SARS]) caused by a novel coronavirus (SARS-CoV) emerged in Guangdong province, China, during November 2002 [1–3]. As a result of international travel, SARS was globally disseminated within months, resulting in >8000 infections and 774 deaths and creating the 21st century's first pandemic [4]. SARS spread to Toronto, Canada, in February 2003, where a subsequent

epidemic resulted in 251 probable cases and 44 deaths, making Toronto the most affected center outside of Asia [4].

Coronaviruses are a diverse family of enveloped, positive-strand RNA viruses that cause a wide spectrum of intestinal and respiratory diseases. For animal coronaviruses, data suggest that changes in the spike glycoprotein may contribute to differences in tissue tropism and virulence [5]. Unlike the enteric and pneumonic illnesses associated with the animal coronaviruses, illnesses associated with previously recognized human coronaviruses (HCoV-229E, HCoV-OC43, and HCoV-NL63) had been largely confined to the upper respiratory tract [6]. However, SARS-CoV appears to have arisen as a result of the zoonotic transmission of an animal coronavirus to humans, and little is known about its potential tissue tropism in humans [6].

Although SARS is primarily characterized by the presence of lower respiratory tract infection and subsequent pulmonary compromise, patients often have evidence of other organ dysfunction, including gastrointestinal symptoms and abnormal liver function [2, 7], as well as splenic atrophy and lymphadenopathy [8]. This may reflect widespread immunopathology or the presence of extrapulmonary SARS-CoV dissemination and replication, as has been observed in other species infected with animal coronavirus [9]. The purpose of the present study was to investigate the presence of SARS-CoV, the degree of viral dissemination, and the viral loads in multiple organ samples from all patients who died of SARS during the Toronto outbreak (March to September 2003) and underwent a postmortem examination and compare the results with those found in patients who died of other causes during the outbreak. We demonstrate that, in fatal cases of SARS, SARS-CoV is often disseminated to multiple organs, in patterns that have implications for clinical manifestations, viral shedding, and disease transmission.

Subjects and methods. All patients whose condition met the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) case definition of probable SARS in Toronto, Canada, and who underwent a postmortem examination during the period from the beginning of the outbreak in March to September 2003 were eligible for inclusion in this study. Autopsies were performed on 21 of the 44 patients with deaths attributed to SARS. Fifteen autopsies were performed using a modified protocol for highly infectious cases, and 6 autopsies were restricted to biopsies of specified tissues.

Fifty-one patients who died of causes other than SARS during the outbreak and who underwent postmortem examination were included as controls. Causes of death in these individuals included congestive heart failure, cerebrovascular accidents, ath-

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Reprints or correspondence: Dr. Kevin Kain, Tropical Disease Unit, Eaton South 9-412, Toronto General Hospital, 200 Elizabeth St., Toronto, Ontario M5G 2C4, Canada (kevin.kain@uhn.on.ca).

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erosclerotic heart disease, chronic obstructive pulmonary disease, invasive group A streptococcal infection, amiodorone pulmonary toxicity, and pulmonary fibrosis.

Clinical details were extracted, by use of hospital records, into standardized data extraction forms. Results of antemortem microbiologic examination for routine bacterial and viral respiratory pathogens from the 21 individuals with probable SARS were negative. However, on the basis of postmortem examination, 3 of these patients had evidence of coinfection with other organisms: 2 women had evidence of coinfection with *Aspergillus* species, and 1 patient had evidence of cytomegalovirus infection. This study was reviewed and approved by the research ethics boards of the Mount Sinai Hospital and the University Health Network, Toronto, Canada, and by the Chief Coroner's Office of Ontario, Canada.

A total of 212 discrete postmortem organ samples, including lung, liver, spleen, kidney, small bowel, large bowel, lymph nodes, heart, and skeletal muscle, were prospectively collected from the 21 patients who died of SARS and underwent autopsies. Two of these patients died >100 days after disease onset, and all organ samples tested were determined by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) to be negative for SARS-CoV. These patients have been excluded from all subsequent analyses. As controls, 228 postmortem organ samples from the same tissues were prospectively obtained from 51 subjects who died of causes other than SARS. All samples collected at the time of autopsy were snap frozen in a mixture of absolute ethanol and dry ice and were subsequently stored at -70°C until analyzed.

All samples were coded and were independently processed and examined. Specimen analysis and interpretation were performed blinded to other diagnostic investigations.

For RT-PCR, organ tissue samples were homogenized, in RLT buffer (Qiagen), by use of disposable tissue grinders (Kendall Precision), and RNA was isolated by use of the RNeasy Mini Kit (Qiagen). The RT-PCR was performed using the commercially available RealArt HPA-Coronavirus LC-RT assay (Artus) on a Lightcycler real-time PCR platform, as described elsewhere [10].

Viral load was calculated from a standard curve based on 4 quantification standards. A standard preparation of SARS-CoV isolated from cell culture supernatants of VeroE6 cells was used as a calibrator in each run. In addition, a second heterologous amplification system (i.e., an internal control) was included to ensure the quality of RNA isolation and to identify PCR inhibition. The sensitivity and specificity of this standardized real-time RT-PCR assay for the detection of SARS-CoV in postmortem lung tissues were previously assessed at 100% [10]. Specificity of amplicons was verified by nucleic acid sequencing.

Results. The 19 patients who died within 51 days after infection and underwent a postmortem examination had a mean age of 68 years (range, 43–99 years). Ten of the patients were

female. The mean duration of illness was 22.5 days (range, 5–51 days).

SARS-CoV was found in 100% (19/19 patients, each with specimens collected from multiple sites, totaling 66 discrete samples) of lung samples, 73% (11/15) of bowel samples, 69% (9/13) of lymph node samples, 41% (7/17) of liver samples, 40% (7/18) of heart samples, 38% (6/16) of kidney samples, and 12% (2/17) of skeletal muscle samples. The viral loads for each organ are presented in tables 1 and 2. Of interest, particularly high viral loads were observed in a recent lung-transplant recipient. All 206 postmortem samples from the 51 non-SARS fatalities that occurred during the SARS outbreak were negative for SARS-CoV. The corresponding sensitivity and specificity of the real-time RT-PCR assay were 100% (95% confidence interval [CI], 94.6%–100%) and 100% (95% CI, 98.2%–100%), respectively, for the detection of SARS-CoV in lung tissue within 51 days of disease onset.

Pathologic findings in SARS-CoV-infected lung specimens showed diffuse alveolar damage; however, histopathologic changes in bowel were minimal, despite the presence of SARS-CoV in >70% of these cases, often at high viral loads (data not shown). There were no changes on gross examination, and only minimal inflammatory changes were observed on microscopic examination. Similarly, despite evidence of elevated antemortem levels of transaminases and the presence of SARS-CoV in the liver at autopsy in 41% of these cases, there were only minor inflammatory changes observed in the liver on microscopic examination. Of note, 100% (7/7) of patients who had SARS-CoV detected in the liver had abnormal antemortem liver-function test results, whereas abnormal results were found in 40% (4/10) who did not have SARS-CoV detected.

Discussion. There are limited data available detailing viral loads and extrapulmonary dissemination of SARS-CoV in humans. In this study, we used a standardized and validated real-time RT-PCR assay to detect and determine the viral load of SARS-CoV in multiple organs from a large number of individuals who died of SARS during the Toronto outbreak, compared with those who died of other causes. We demonstrate that, even 51 days after disease onset, SARS-CoV was consistently found in multiple lung lobes, suggesting that there is widespread dissemination of the virus throughout the lung at time of death. Further, we observed extrapulmonary dissemination of the virus into all major organs, especially the bowel and lymph nodes. These data have implications for the clinical manifestation, disease course and outcome, and transmission of SARS-CoV.

SARS-CoV viral dissemination has previously been examined in a simian model [11]. Necropsy results indicated the widespread presence of SARS-CoV in lung but only sporadic presence of the virus in the duodenum, kidney, and spleen. Although our findings that SARS-CoV is detected in postmortem

Table 1. Clinical description and severe acute respiratory syndrome (SARS) coronavirus (CoV) dissemination to multiple organs in 19 patients who died of probable SARS.

Patient no. (sex/age in years)	Comorbidities	Illness or treatment duration, days				Postmortem tissue SARS CoV RT-PCR results ^a								
		Illness	Ventilation	Ribavirin	Steroids ^b	Lung	Liver	Spleen	Kidney	Small bowel	Large bowel	Lymph nodes	Heart	Muscle
A109 (F/88)	HTN, CVD, osteoporosis	5	DNI	0	0	1.0×10^{10}	NA	NA	NA	NA	NA	NA	1.6×10^4	–
A53 (M/62)	Rectal cancer, HTN, hypercholesterolemia	10	5	3	4	5.3×10^7	6.0×10^3	1.3×10^4	–	2.7×10^9	5.9×10^4	NA	2.6×10^4	2.8×10^4
A108 (F/67)	None	10	0	0	2	3.3×10^5	NA	NA	NA	NA	NA	NA	1.0×10^4	–
A52 (M/76)	Type 2 DM, CAD, HTN	11	4	4	0	3.8×10^9	1.8×10^4	7.2×10^5	3.7×10^4	NA	NA	NA	–	NA
A113 (M/57)	DBL LT for COPD and BOOP, CVD, post LT DM, atrial fibrillation	14	6	0	6	8.8×10^9	1.6×10^6	1.4×10^5	7.4×10^5	2.4×10^9	3.7×10^9	8.9×10^9	2.8×10^7	1.0×10^4
A51 (F/78)	Type 2 DM, CAD, COPD, hypercholesterolemia	15	5	5	0	1.1×10^9	2.8×10^4	2.1×10^5	1.2×10^5	7.3×10^4	1.3×10^5	NA	3.5×10^5	–
A39 (M/43)	Type 2 DM, HTN	16	4	0	0	6.4×10^9	6.0×10^3	–	NA	NA	NA	NA	NA	NA
A71 (M/78)	Type 2 DM, HTN, hypercholesterolemia	≥18	≥4	>2	>7	5.7×10^5	–	–	–	2.7×10^5	5.1×10^4	9.5×10^4	–	–
A67 (M/63)	Hypercholesterolemia, CVD	20	12	16	14	2.5×10^7	–	6.1×10^4	–	3.3×10^5	6.3×10^6	2.8×10^4	3.2×10^4	–
A68 (F/78)	Type 2 DM, HTN, hypercholesterolemia	23	18	11	18	4.9×10^5	5.0×10^4	3.6×10^4	5.9×10^4	4.7×10^6	7.7×10^7	4.2×10^5	6.8×10^4	–
A57 (F/73)	HTN, hypercholesterolemia	24	DNI	8	11	3.6×10^5	–	–	–	–	6.1×10^5	1.5×10^4	–	–
A154 (M/45)	None	24	12	0	10 (+ IFN 10)	5.5×10^4	1.8×10^4	8.7×10^3	4.3×10^3	2.7×10^3	5.3×10^3	7.1×10^4	–	–
A63 (F/99)	Osteoarthritis	25	DNI	7	0	5.0×10^4	–	–	–	1.7×10^4	6.1×10^4	–	–	–
A159 (F/81)	Spinal tumor	27	24	0	18 (+ IFN 11)	2.0×10^4	–	1.3×10^4	8.9×10^3	1.2×10^4	2.9×10^5	7.0×10^4	–	–
A69 (M/44)	None	29	18	18	17	7.6×10^4	–	4.8×10^4	–	9.2×10^5	–	6.1×10^5	–	–
A72 (F/79)	Type 2 DM, HTN, hypercholesterolemia	29	DNI	7	9	2.1×10^5	–	–	–	1.3×10^4	–	–	–	–
A150 (M/68)	CVD	34	DNI	NA	NA	2.4×10^4	–	–	–	–	–	2.0×10^4	–	–
A164 (F/51)	Borderline DM	43	31	0	22 (+ IFN 20 and IVIG)	1.5×10^5	–	–	–	–	1.4×10^4	–	–	–
A173 (F/77)	Asthma, HTN	51	43	0	5	2.9×10^5	–	–	–	–	–	–	–	–

NOTE. BOOP, bronchiolitis obliterans and organizing pneumonia; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CVD, cerebral vascular disease; DBL LT, double lung transplant; DM, diabetes mellitus; DNI, do not intubate; HTN, hypertension; IFN, interferon; IVIG, intravenous immunoglobulin; NA, not available.

^a Positive results are denoted by their SARS CoV levels, in copies/g; minus signs (–) denote negative results.

^b Supplementary treatments and their duration in days are shown in parentheses.

Table 2. Systemic distribution of severe acute respiratory syndrome coronavirus and corresponding viral loads.

Organ	Maximum viral load, copies/g of tissue	Median viral load, copies/g of tissue	Patients with virus detected in organ, % (no./total)
Lung	1.0×10^{10}	3.6×10^5	100 (19/19)
Small bowel	2.7×10^9	2.7×10^5	73 (11/15)
Large bowel	3.7×10^8	1.3×10^5	73 (11/15)
Lymph nodes	8.9×10^8	7.1×10^4	69 (9/13)
Spleen	7.2×10^5	4.8×10^4	53 (9/17)
Liver	1.6×10^6	1.8×10^4	41 (7/17)
Heart	2.8×10^7	3.2×10^4	40 (7/18)
Kidney	7.4×10^5	4.8×10^4	38 (6/16)
Skeletal muscle	2.8×10^4	2.8×10^4	12 (2/17)

human lung tissue correspond to those of Kuilken et al., their observation that virus was only sporadically identified in other organs differs from our observations in fatal human infection. Kuilken et al. attribute this sporadic detection to overspill from other tissues, perhaps via blood, but do not comment on the SARS-CoV viral load in any tissue. Our findings suggest extrapulmonary SARS-CoV dissemination and demonstrate comparable viral loads in small bowel, large bowel, and lymph nodes. These data, combined with evidence of viral shedding in stool and urine, viral replication in the gut, and reports of transient viremia and relatively low viral loads in the blood, suggest that viral overspill is perhaps a less likely explanation for the extrapulmonary dissemination of SARS-CoV that we observed in humans [12].

Of interest, angiotensin-converting enzyme 2 (ACE2), the putative functional receptor of SARS-CoV [13], is expressed in many of the organs in which we observed SARS-CoV dissemination, including the gastrointestinal tract, heart, kidney, lung, lymph nodes, skeletal muscle, liver, and spleen [14].

Gastrointestinal complaints—and watery diarrhea, in particular—are frequent symptoms of SARS, reported at presentation by $\geq 20\%$ of infected individuals [12] and developing in as many as 70% of individuals during the course of illness [15]. In this study, we demonstrated that the majority of patients had evidence of SARS-CoV in both large and small bowel, often at high viral loads. These data suggest that SARS-CoV displays tissue tropism for the bowel and provide a putative mechanism for the frequent occurrence of gastrointestinal symptoms in this population. This hypothesis is supported by observations that SARS-CoV is frequently and persistently identified in fecal specimens [1, 2, 11] and by recent electronic microscopic evidence indicating viral replication and recovery of SARS-CoV from postmortem small-bowel specimens [12]. Collectively, these observations indicate that enteric involvement is common in SARS and have implications for infection-control measures and the potential for fecal-oral transmission in community outbreaks.

Evidence of hepatic dysfunction is common in SARS, and

elevated serum alanine aminotransferase levels are observed before death in $\geq 40\%$ of patients [6]. In this study, we observed a relationship between the presence of SARS-CoV in the liver and antemortem abnormal liver function tests, supporting a role for SARS-CoV in mediating hepatic dysfunction. Furthermore, splenic atrophy and lymphadenopathy with tissue necrosis have also been reported in SARS [2, 7, 8]. These findings can potentially be explained by our observations of elevated viral loads at these sites. Ding et al. hypothesized that the degenerative changes observed in these organs are most likely due to disturbed cell metabolism caused by rapid viral replication [8], but the authors lacked the viral-load evidence we present here to support these contentions. Although organ damage in patients with SARS may be the immunopathologic consequence of an exuberant host response [2], evidence of elevated viral loads and of extrapulmonary viral dissemination suggests that regional viral replication could also potentially contribute to organ dysfunction and exacerbate the host response at these sites.

Although SARS-CoV has previously been detected in urine and stool [1, 2, 11] from patients with SARS, to our knowledge there is only 1 report of SARS-CoV detection in kidney [3]. In addition, Kuiken et al. found PCR evidence of SARS-CoV in kidney, duodenum, and stomach in 1 of 4 SARS-CoV-infected macaques, but no viral loads were given [11]. Our findings of high viral loads in the gut and liver and moderate viral loads in the kidney are consistent with previous reports of elevated viral shedding in the stool and moderate to low shedding in urine [1, 2].

The current findings provide insight into multiorgan SARS-CoV dissemination and viral load at the time of death, on the basis of a prospective and systematic examination of a large number of fatal SARS cases. SARS-CoV was consistently identified in the lungs of patients who died of SARS and was not found in control individuals, supporting a direct role for SARS-CoV in contributing to fatal outcomes. SARS-CoV disseminates to other organs, which may explain, at least in part, the clinical manifestations and pattern of viral shedding observed in SARS-

CoV-infected patients and may provide insight into the selection of appropriate clinical samples in the event of a future outbreak.

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