Analysis of Deaths During the Severe Acute Respiratory Syndrome (SARS) Epidemic in Singapore

Challenges in Determining a SARS Diagnosis

Pek Yoon Chong, MBBS, FRCPA; Paul Chui, MBBS, MRCPath (Forensic); Ai E. Ling, MBBS, FRCPA; Teri J. Franks, MD; Dessmon Y. H. Tai, MBBS, FCCP; Yee Sin Leo, MBBS; Gregory J. L. Kaw, MBBS, FRCR; Gervais Wansaicheong, MBBS, FRCR; Kwai Peng Chan, MBBS; Lynette Lin Ean Oon, MBBS, FRCPA; Eng Swee Teo, MBBS; Kong Bing Tan, MBBS; Noriko Nakajima, MD; Tetsutaro Sata, MD; William D. Travis, MD

● ***Context.*—An outbreak of severe acute respiratory syndrome (SARS), an infectious disease attributed to a novel coronavirus, occurred in Singapore during the first quarter of 2003 and led to 204 patients with diagnosed illnesses and 26 deaths by May 2, 2003. Twenty-one percent of these patients required admission to the medical intensive care unit. During this period, the Center for Forensic Medicine, Health Sciences Authority, Singapore, performed a total of 14 postmortem examinations for probable and suspected SARS. Of these, a total of 8 were later confirmed as SARS infections.**

***Objective.*—Our series documents the difficulties encountered at autopsy during the initial phases of the SARS epidemic, when the pattern of infection and definitive diagnostic laboratory criteria were yet to be established.**

***Design.*—Autopsies were performed by pathologists affiliated with the Center for Forensic Medicine, Health Sciences Authority, Singapore. Tissue was accessed and read at the Tan Tock Seng Hospital, Singapore, and at the Armed Forces Institute of Pathology, Washington, DC. Autopsy tissue was submitted to the Virology Department, Singapore General Hospital, for analysis, and in situ hybridization for the SARS coronavirus was carried out at the National Institute of Infectious Diseases, Tokyo, Japan.**

***Results.*—Thirteen of 14 patients showed features of diffuse alveolar damage. In 8 patients, no precipitating etiology was identified, and in all of these patients, we now have laboratory confirmation of coronavirus infection. Two of the 8 patients presented at autopsy as sudden unexpected deaths, while the remaining 6 patients had been hospitalized with varying lengths of stay in the intensive care unit. In 3 patients, including the 2 sudden unexpected deaths, in situ hybridization showed the presence of virally infected cells within the lung. In 4 of the 8 SARS patients, pulmonary thromboemboli were also recognized on gross examination, while one patient had marantic cardiac valvular vegetations.**

***Conclusions.*—It is unfortunate that the term *atypical pneumonia* has been used in conjunction with SARS. Although nonspecific by itself, the term does not accurately reflect the underlying dangers of viral pneumonia, which may progress rapidly to acute respiratory distress syndrome. We observed that the clinical spectrum of disease as seen in our autopsy series included sudden deaths. This is a worrisome finding that illustrates that viral diseases will have a spectrum of clinical presentations and that the diagnoses made for such patients must incorporate laboratory as well as clinical data.**

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Severe acute respiratory syndrome (SARS), an infectious disease attributed to a novel coronavirus,[1,2] was imported into Singapore in March 2003 by a patient who had traveled to Hong Kong[3] and who had stayed in the same hotel and same floor as the physician from Guangdong who is believed to be the source of infection for the outbreak in Canada.[4] This index case subsequently led to an outbreak of SARS in Singapore, with a total of 204 infections (103 of which were infected by 5 sources[5]) and 26 deaths by the beginning of May.[6]

Singapore had 4 clusters of infections during the 2-month period from mid March to the beginning of May.[3] Three were centered on hospitals (Tan Tock Seng Hospital with 1163 beds, Singapore General Hospital with 1502 beds, and National University Hospital with 943 beds[7]), while a fourth group developed around a vegetable wholesale center at Pasir Panjang, Singapore.

Approximately 75% of the SARS infections in Singapore have been traced to hospitals, and about 41% of the SARS

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From the Department of Pathology and Laboratory Medicine (Dr Chong), the Medical ICU (Dr Tai), and the Department of Diagnostic Radiology (Drs Kaw and Wansaicheong), Tan Tock Seng Hospital, Singapore; the Center for Forensic Medicine, Health Science Authority, Singapore (Drs Chui and Teo); the Virology Department, Singapore General Hospital, Singapore (Drs Ling, Chan, and Oon); the Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, Washington, DC (Drs Franks and Travis); the Communicable Diseases Center, Singapore (Dr Leo); the National University of Singapore, Singapore (Dr Tan); and the Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan (Drs Nakajima and Sata). Dr Chong is now with the Mount Elizabeth Hospital, Parkway Laboratory Services Ltd, Singapore.

Reprints: P. Y. Chong, MBBS, FRCPA, Mount Elizabeth Hospital, Parkway Laboratory Services Ltd, 3 Mount Elizabeth, Singapore 228510 (e-mail: pychong@gleneagles.com.sg).

Table 1. Correlation Between Clinical Diagnosis and Main Pathology Findings*

Case No.	Sex/ Age	Clinical Diagnosis	Right Lung, g	Left Lung, g	External Examination	Others
			Confirmed SARS Cases			
1	M/50 y	SARS (P), multiple organ failure	1507	1497	Multiple hemorrhagic infarcts, both lungs; subpleural hemorrhages	Organizing thrombi with occlusion of segmental pulmonary arteries, lungs; deep vein thrombosis; corticosteroid therapy, 9 d; mechanical ventilation, 4 d
2	M/39 y	SARS (P), acute myocardial infarct, septic shock	1367	1194	Multiple hemorrhagic infarcts, both lungs; multiple subpleural hemorrhages; red and gray hepatization	Organizing thrombi with occlusion of segmental pulmonary arteries, lungs; marantic vegetations: tricuspid, mitral, and aortic valves plus systemic infarcts; deep vein thrombosis; corticosteroid therapy, 9 d; mechanical ventilation, 8 d
3	F/29 y	SARS (P), septic shock, multiple organ failure	1034	930	Hemorrhagic and pale infarcts, both lobes; gray hepatization	Fragmented thrombi with occlusion of segmental pulmonary arteries, lungs; focal nodular hyperplasia, liver; deep vein thrombosis; thrombosis of paraovarian and parauterine veins; corticosteroid therapy, 3 d; mechanical ventilation, 6 d
4	M/68 y	Suspected SARS (S), acute myocardial infarct, hypertension, diabetes mellitus, end-stage renal failure on hemodialysis, ischemic heart disease, gout	841	666	Hypostatic congestion and consolidation; no infarct seen	Subendocardial infarct with coronary occlusive disease (75% occlusion); mechanical ventilation, <1 d
5	M/63 y	SARS (P), multiple organ failure, ischemic heart disease, congestive cardiac failure	1254	1074	Firm with multiple small cystic spaces, 5 mm each; no infarct	Coronary vessels: 10% occlusion; nephrosclerosis with infarct; mechanical ventilation, 2 d
6	M/38 y	Unable to exclude SARS (S), bilateral pneumonia, hypertension	1316	1179	Meaty, reddish appearance with scattered subpleural hemorrhages	Nonocclusive pulmonary artery thrombus, 4.5 cm; mechanical ventilation, <1 d
7	F/67 y	Unable to exclude SARS (S), fever, BID	770	580	Firm, patchy, red hepatization; severe congestion	
8	M/43 y	Unable to exclude SARS (S), ? dengue, ? upper respiratory tract illness	750	650	Diffuse consolidation; severely congested	
			Non-SARS Cases			
9	F/16 mo	Unable to exclude SARS (S), viral pneumonitis, status asthmaticus	318	261	Diffuse reddish appearance, whitish speckling; subpleural pale areas	Nil of note; mechanical ventilation, 2 d
10	M/87 y	Unable to exclude SARS (S), nosocomial pneumonia, sepsis, cardiac failure, ischemic heart disease	1309	434	Gray hepatization with nodular areas	Iliac artery aneurysm; hypertensive nephrosclerosis; mechanical ventilation, 4 d
11	M/47 y	Probable SARS (P), multiple organ failure, septic shock, DIVC	1689	1158	Multiple subpleural hemorrhagic subpleural infarcts with central softening; gray hepatization	Fibrin thrombi within glomeruli; mechanical ventilation, 2 d
12	M/70 y	Probable SARS (P), multiple organ failure, intra-abdominal abscess, hypertension, gout, acute renal failure, diabetes mellitus	616	604	Bronchopneumonia	Perforated duodenum; hypertensive and diabetic nephrosclerosis; mechanical ventilation, >7 d
13	M/88 y	Unable to exclude SARS (S), nosocomial pneumonia, ARDS, multiple fractures	460	788	Congestion with pneumonia and small 5-mm cavities	Multiple fractures; hypertensive nephrosclerosis; mechanical ventilation, 7 d
14	M/47 y	Unable to exclude SARS (S), fever, BID	934	813	Multiple pleural petechial hemorrhages	Nil of note

* SARS indicates severe acute respiratory syndrome; BID, brought in dead; DIVC, disseminated intravascular coagulation; and ARDS, acute respiratory distress syndrome. SARS status is indicated as probable (P) or suspect (S).

patients were health care workers. In our experience, 21% (45) of the SARS patients required admission to the medical intensive care unit (ICU) and, of these, 84% required intubation for acute lung injury ($P_{AO_2}/F_{IO_2} \leq 300$) or acute respiratory distress syndrome (ARDS) ($P_{AO_2}/F_{IO_2} \leq 200$).[8]

During this period, the Center for Forensic Medicine, Health Sciences Authority, Singapore, performed 14 autopsies (Table 1) from different hospitals in Singapore, 6 of which were for probable SARS cases and 8 of which were for suspected SARS cases. Nine of these were mandated by the Director of Medical Services, Singapore, under the

Table 2. Clinical Profile of Autopsy Cases*

	SARS	Non-SARS
Sex: male-female ratio	6:2	5:1
Age: mean (range)	50 y (29–68)	56 y (1.5–88)
Length of hospital stay	6.1 d (0–11)	11.5 d (0–40)
Comorbid factors	Diabetes mellitus, end-stage renal failure, ischemic heart disease, hypertension	Diabetes mellitus, hypertension, multiple fractures, sepsis, empyema gall bladder, asthma, HIV
Symptoms		
Fever, mean (range)	37.6°C (35–38.9)	39°C (37.8–40.5)
Cough	4/8 patients	3/6 patients
Shortness of breath	4/8	2/6
Myalgia	3/8	0/6
Diarrhea	2/8	0/6
Chest pain	0	1/6
Others	Headache, giddiness, runny nose	Uncontrolled diabetes, road traffic accident, abdominal pain
Chest x-ray film, initial changes†	Normal, 1/6 patients; patchy ground-glass opacities, 5/6	Reticular linear shadows, 1/5; lobar consolidation, 1/5; normal, 2/5; fractured ribs, 1/5

Hematology Component (Reference Range)	SARS, Mean (Range)‡		Non-SARS (Based on 3 Patients Admitted Immediately to the ICU), Mean (Range)§
	Hospital	Medical ICU	
Total white blood cell count (4–10 × $10^3/\mu L$)	7.5 (66–88.5)	10.9 (5.4–14)	8.5 (5–11.8)
Neutrophils (40%–70%)	76.55 (66–88.5)	85.4 (67–93)	71.2 (47–86.6)
Lymphocytes (18%–43%)	15 (10–24)	10.6 (5.8–32.8)	18.4 (4–44)
Platelets (160–390 × $10^3/\mu L$)	173 (92–287)	232 (70–430)	108 (53–199)
LDH (180–380 U/L) (in 3 patients)	845 (649–1125)	1434 (748–2101)	597 (only 1 patient, case 11)

* SARS indicates severe acute respiratory syndrome; HIV, human immunodeficiency virus; ICU, intensive care unit; and LDH, lactate dehydrogenase.

† In 6 SARS patients and 5 non-SARS patients on admission; 2 SARS patients and 1 non-SARS patient were not admitted.

‡ In 6 SARS patients, on admission to the hospital and on admission to the ICU.

§ Not including case 9, the pediatric case, and case 12, the uncontrolled diabetic patient with multiple episodes in the ICU.

Infectious Diseases Act.[9] Three of the 14 cases presented as sudden unexpected deaths at the forensic service, 2 of which were later classified as deaths due to SARS.

MATERIALS AND METHODS

All autopsies were carried out by pathologists (P.C., E.S.T., and K.B.T.) at the Health Sciences Authority, Singapore. Twelve were performed using positive air purifying respirators, and 2 were performed using routine precautions currently in place with N95 masks, disposable visors, gloves, protective gowns, and aprons.

Postmortem tissue was sent to the Tan Tock Seng Hospital, Singapore, and the Armed Forces Institute of Pathology, Washington, DC, for histopathology. Stains for macrophages and lymphocytes (CD68 and CD3, Dako Cytomation, Glostrup, Denmark) were also performed using the Ventana detection kit (Ventana Medical Systems, Tucson, Ariz).

Tissue was taken at autopsy for immunofluorescent antigen detection of respiratory viruses using the following methods: (1) polymerase chain reaction for coronavirus with a range of primers, including SAR1a/as and BNIoutS2/As2[2] and Cor1/2 (sense 5′-CAC CGT TTC TAC AGG TTA GCT AAC GA-3′ and antisense 5′-AAA TGT TTA CGC AGG TAA GCG TAA AA-3′) from the Government Virus Unit, Hong Kong,[10] and (2) viral isolation on a variety of cell lines, including Vero cells. Immunoglobulin M and G (IgM and IgG) antibodies to the coronavirus were detected using an immunofluorescent antibody assay. These assays were performed in the Virology Department, Singapore General Hospital (A.E.L., K.P.C., and L.L.E.O.).

In an attempt to localize the virus, unstained sections of the lung from the 8 cases with a positive polymerase chain reaction for the SARS coronavirus were submitted to the National Institute of Infectious Diseases, Tokyo, Japan, for detection of SARS using the recently described in situ hybridization-AT tailing technique with catalyzed signal amplification (T.S. and N.N.).[11]

Data from the Tan Tock Seng Hospital were collated by the Department of Diagnostic Radiology (G.J.L.K. and G.W.), the Medical ICU (D.Y.H.T.), and the Communicable Disease Center (Y.S.L.).

RESULTS

Table 1 summarizes the clinical diagnoses and significant pathology findings with special reference to the pulmonary system in SARS and non-SARS cases. Table 2 gives the clinical profile of the SARS patients, and Table 3 details the virology and other microbiology findings in SARS and non-SARS cases. Table 4 gives details of the in situ hybridization-AT tailing–catalyzed signal amplification staining in the SARS cases.

Clinical Findings

Eleven inpatients with progressive pulmonary symptoms subsequently underwent an autopsy for a clinical diagnosis of SARS or suspected SARS. Nine either had a history of exposure to SARS or had traveled to an affected area. Two (patients 9 and 11) were referred for autopsy because of a short history of fever, a rapid progression of disease, and radiologic findings that were suggestive of SARS. Three were routine coroner autopsies at which the forensic pathologist thought the pulmonary findings were suggestive of ARDS[12] but for which there was no apparent risk factor.

Eight patients (6 males and 2 females; mean age, 50 years) subsequently had laboratory confirmation of SARS infections, while 6 patients (5 males and 1 female; mean age, 56 years, including a pediatric patient, or 67 years, if the pediatric patient was excluded) were negative for coronavirus. Both groups had patients with comorbid conditions (Table 2).

Table 3. Virology and Microbiology Data*

Case No.	Autopsy Tissue Tested PCR Positive for Coronavirus†	Culture for Coronavirus	IFA Serology for Coronavirus	IF and Isolation for Respiratory Viruses‡	Microbiology Results
			SARS Cases		
1	Lung§	Negative	IgM, IgG seroconversion, 12-d interval	Negative	*Pseudomonas aeruginosa*
2	ETT aspirate, lung§	Negative	IgM, IgG seroconversion, 11-d interval	Negative	Methicillin-resistant *Staphylococcus aureus*
3	Lung	Negative	IgM seroconversion, 8-d interval	Negative	No growth
4	Tracheal swab, spleen, brain, heart, lymph node, lung, and liver	Positive in all except liver	Not done	Negative	No growth
5	Intestine, lymph node, spleen, lung	Positive only in lung	Negative, 6 d apart	Negative	No growth
6	Tracheal swab, heart, lung	Negative	Not done	Negative	No record
7	Heart and lung	Positive	Not done	Negative	α-Hemolytic *Streptococcus* sp, *Klebsiella* sp
8	Heart and lung	Positive	Not done	Negative	No record
			Non-SARS Cases		
9	Negative	Negative	Not done	Culture positive for adenovirus (ETT aspirate, conjunctival swab, intestine, and lung)	No growth
10	Negative	Negative	Not done	Negative	*Pseudomonas aeruginosa, Candida tropicalis, Candida glabrata*
11	Negative	Negative	Not done	Negative	*Streptococcus pneumoniae*
12	Negative	Negative	Negative, 6 d apart	Negative	*Acinetobacter baumanii, Enterococcus* sp, methicillan-resistant *Staphylococcus aureus*
13	Negative	Negative	Not done	Negative	*S pneumoniae, Enterobacter* sp
14	Negative	Negative	Not done	Negative	No record

* SARS indicates severe acute respiratory syndrome; PCR, polymerase chain reaction; IFA, immunofluorescent assay; IF, immunofluorescence; IgM and IgG, immunoglobulins M and G; and ETT, endotracheal tube.
† Autopsy tissue taken for virology testing includes ETT swabs/aspirates, lung, lymph node, spleen, brain, heart, liver, and intestine.
‡ IF testing for respiratory viruses includes respiratory syncytial virus; parainfluenza 1, 2, and 3; influenza A and B; and adenovirus.
§ PCR testing by Defense Medical Research Laboratory.

Table 4. In Situ Hybridization (ISH) Results in Severe Acute Respiratory Syndrome (SARS) Cases*

Case No.	DAD Acute	DAD Organizing	Bronchus
1	Negative	Negative	Negative
2	Negative	Negative	Negative
3	Negative	Negative	NA
4	Positive	Positive	Negative
5	Negative	Negative	Negative
6	Negative	Negative	Negative
7	Positive	NA	NA
8	Positive	NA	NA

* ISH-AT tailing–catalyzed signal amplification of lung from the 8 SARS cases using antisense probe to the nucleocapsid protein of the SARS coronavirus. DAD indicates diffuse alveolar damage; NA, not available for testing.

The clinical course for the 8 SARS patients of our study varied (see Figures 1 through 5). Patients 1, 2, and 3 stayed a mean average of 10 days (range, 10–13) in the hospital, received mechanical ventilation for 7 days (range, 4–8), and died about 3 weeks into their illness. Patients 4 and 5 died within 5 to 8 days of the detection of fever. Patient 4 had significant comorbid factors such as end-stage renal failure and ischemic heart disease. His last admission was for an elevated temperature of 38.5°C. Patient 5 had hypertension and was admitted with a temperature of 35°C and a blood pressure level of 80/50 mm Hg. Patient 6 was febrile, deteriorated rapidly for 2 days, and collapsed 10 days after the onset of fever. Patient 8 saw his general practitioner twice for 1 week for fever (37.7°C) but collapsed at home on day 8. The most rapid onset documented was in case 7, in which the patient had visited a physician 2 days before she died with complaints of fever and a runny nose. Her temperature was recorded on the 2 days preceding her death as 38°C and, without the use of antipyretics, as 37.4°C.

Virology

Virology data are summarized in Table 3. Tissue from the lung yielded positive polymerase chain reaction results in all 8 patients, while heart tissue was positive in 4 of 8 patients. The SARS coronavirus was successfully iso-

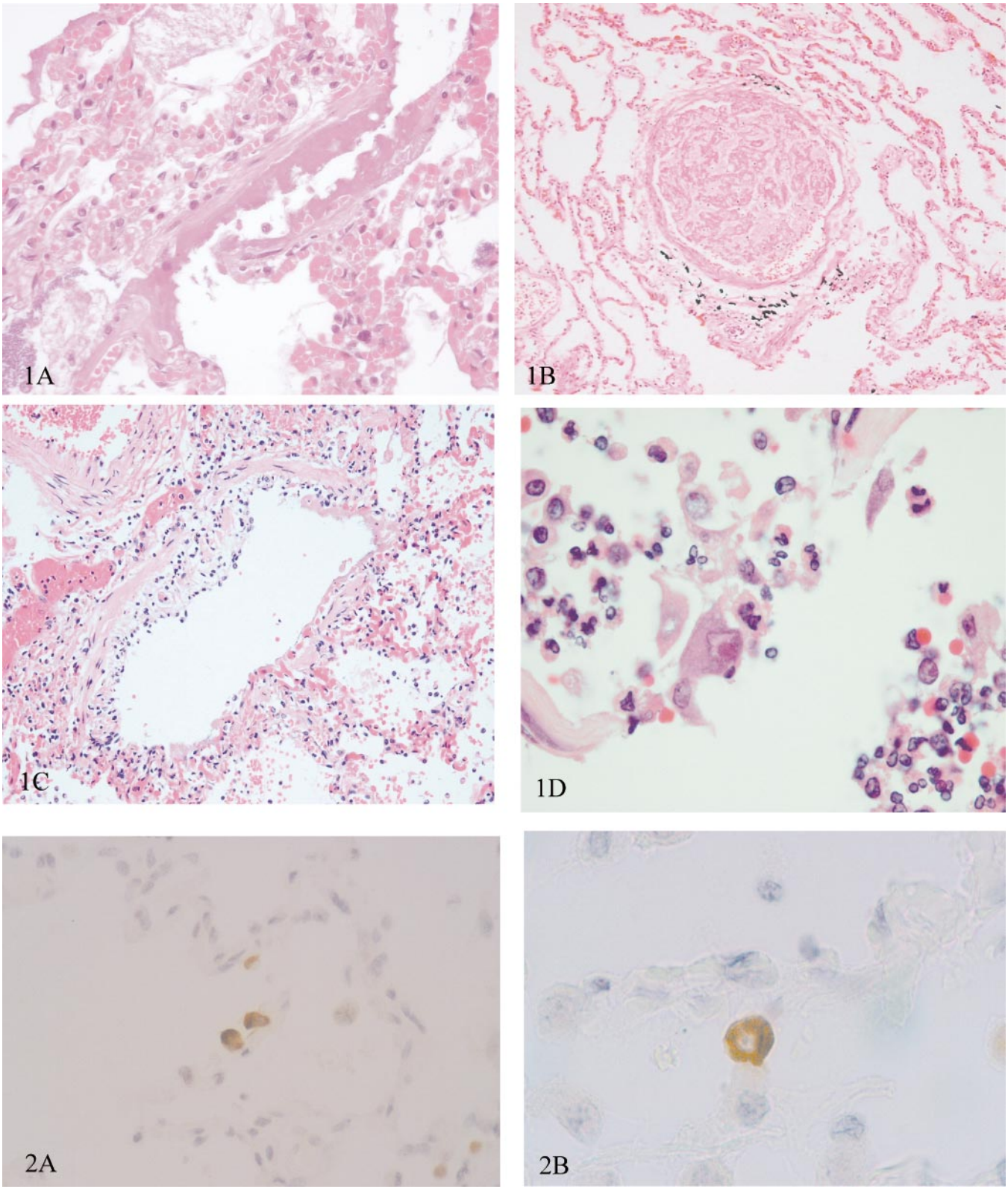

Figure 1. *Diffuse alveolar damage in severe acute respiratory syndrome. A, Acute-phase diffuse alveolar damage from case 7 (hematoxylin-eosin, original magnification ×400). B, Fibrin thrombi in acute-phase diffuse alveolar damage, case 8 (hematoxylin-eosin, original magnification ×200). C, Necrosis of bronchiole mucosa, case 6 (hematoxylin-eosin, original magnification ×200). D, Atypical epithelial cell, case 3 (hematoxylin-eosin, original magnification ×1000).*

Figure 2. *A and B, In situ hybridization for severe acute respiratory syndrome coronavirus, case 7. Positive signal located in the cytoplasm of cells lining the alveolar walls (antisense NP probe, AT tailing amplification, diaminobenzidine chromagen with hematoxylin counterstain, original magnifications ×400 [A] and ×1000 [B]).*

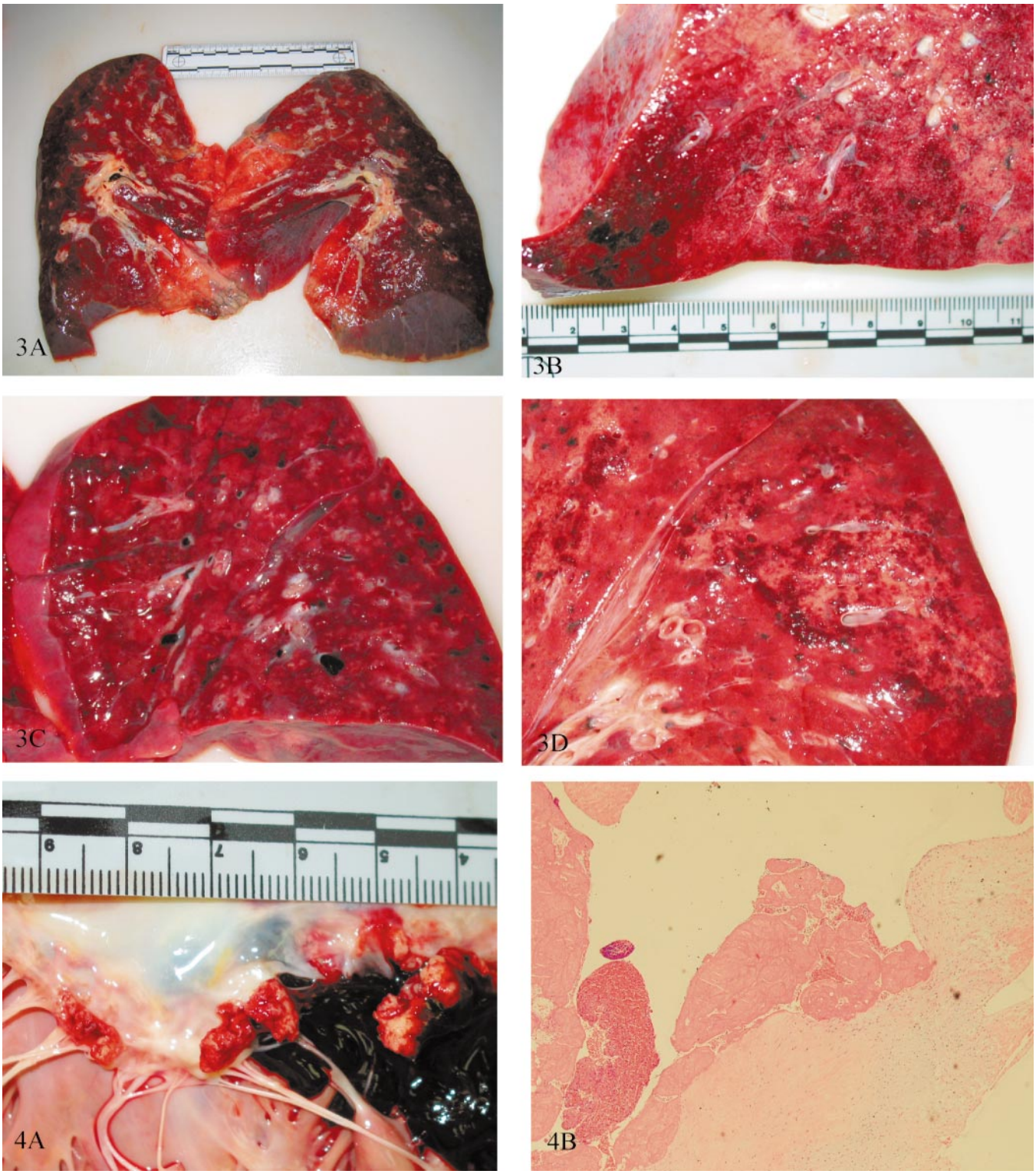

Figure 3. *Gross appearance of lung with diffuse alveolar damage from severe acute respiratory syndrome (SARS) and non-SARS cases at autopsy. A, SARS lung from case 4 showing reddish, meaty appearance with poor aeration. B, SARS lung from case 2, showing a grayish, solid, meaty appearance with hemorrhagic infarct. C, Adenovirus pneumonia, case 9, showing small, pale nodules distributed diffusely through the lung parenchyma. D, Streptococcal pneumonia, case 11 with a septic infarct.*

Figure 4. *Multiple pathology in severe acute respiratory syndrome. A, Case 2 had large, friable vegetations along the valve edge. B, Light microscopy confirms the presence of fibrin (hematoxylin-eosin, original magnification ×40).*

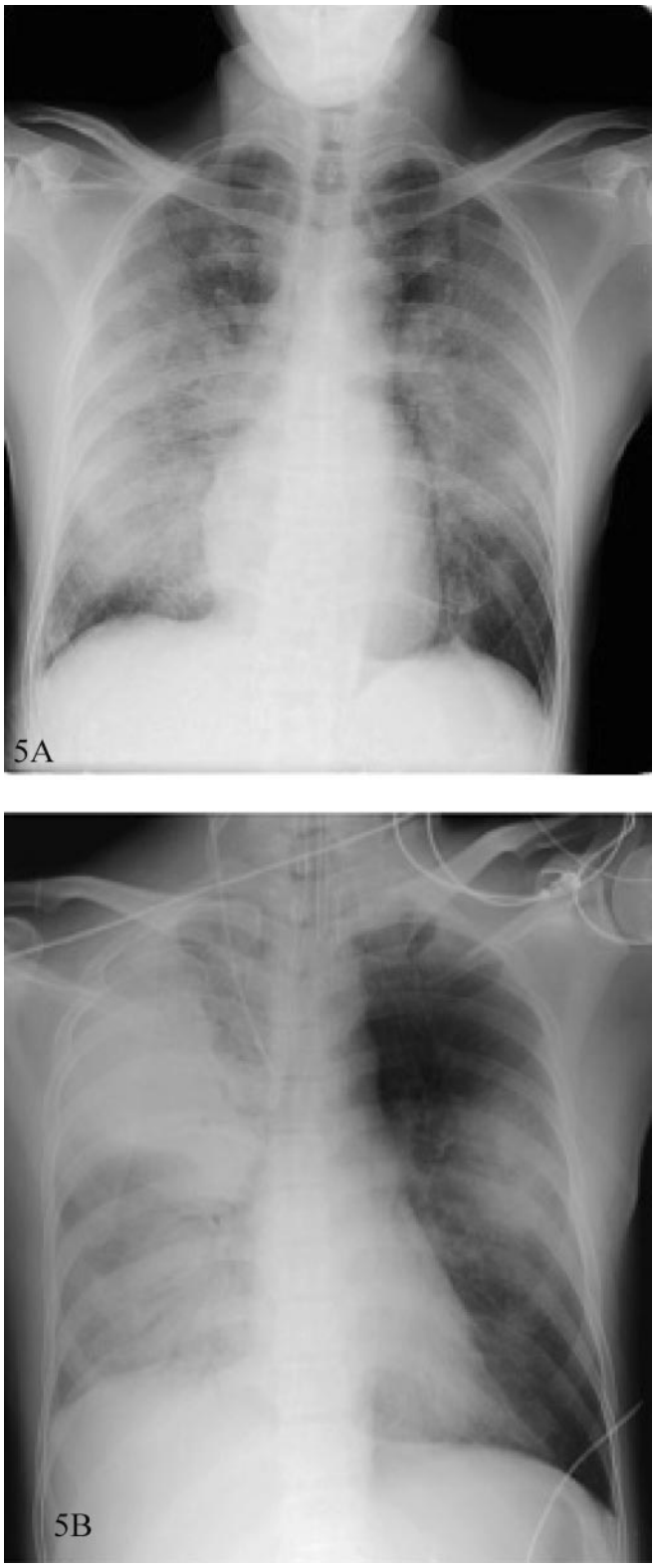

Figure 5. *Chest radiographs in severe acute respiratory syndrome (SARS) and non-SARS. A, Chest radiograph in more advanced stages of a SARS coronavirus infection showing bilateral, diffuse, ground-glass shadowing with consolidation at the periphery of both lungs. B, Chest radiograph from a non-SARS case showing lobar consolidation involving the right upper and lower lobes with air bronchograms. A focus of consolidation is seen at the left middle zone.*

lated from patients 4, 5, 7, and 8, and the adenovirus was isolated from patient 9.

Patients 1, 2, 3, 5, and 12 had an immunofluorescent assay for antibodies to the coronavirus. Of these, patients 1, 2, and 3 showed seroconversion from negative to positive during an 8- to 12-day period. Patients 5 and 12 did not show seroconversion; however, the paired sera available for assay were collected during a shorter 6-day interval, during which seroconversion may not have occurred.

Epidemiology

Nine of 14 patients were known to have had possible exposure to SARS. Patients 1 and 2 were family members or friends of Singapore's first index case/patient. Patient 3 was a separate imported case with a history of travel to Hong Kong. Six patients (4, 5, 6, 10, 12, and 13) were linked to hospitals, while 5 patients (7, 8, 9, 11, and 14) did not have definite links at the time of autopsy.

Radiology

Plain chest x-ray films were available for review at autopsy from 9 of 11 patients. The 3 patients with sudden unexpected death had received no prior radiologic examination.

In the radiographs of 6 of the SARS patients, the early changes were seen as patchy ground-glass opacities, distributed mainly in the central and peripheral areas of the middle and lower zones of the lungs (6 of 6 patients). In 5 patients, these changes were seen in the initial x-ray films. In the non-SARS cohort, the initial x-ray films showed a bilateral reticular interstitial pattern (patient 9), a lobar consolidation in the right lung (patient 11), and a normal chest radiograph (patient 12).

In the SARS cohort, the later radiographs showed bilateral, symmetric, diffuse, ground-glass opacities with patchy consolidation.

Pathology

Pulmonary.—The main pathology in these 14 cases concerned the pulmonary system. All of the patients had heavy lungs that were firm and poorly aerated with minimal edema. Seven of the 14 patients had lung weights greater than 1000 g per lung. The appearance of the patients' lungs at autopsy is described in Table 1.

Thirteen of the 14 patients had diffuse alveolar damage on light microscopy. Of the non-SARS cases, 2 had known risk factors[13–15] for diffuse alveolar damage, such as trauma (patient 13) and surgery with septicemia (patient 12). In 3 patients, classic patterns of infections known to be associated with hyaline membrane formation,[13] such as adenovirus pneumonia (patient 9), *Streptococcus pneumoniae* (patient 11), and fulminant *Pneumocystis carinii* pneumonia (patient 14), were seen.

Patient 10 developed respiratory distress following aortofemoral bypass surgery. The differential diagnosis considered clinically was between a nosocomial infection and SARS, as he had been exposed to the infection during surgery. At autopsy, however, he had asymmetrically heavy lungs (right lung, 1309 g; left lung, 434 g), which suggested lobar pneumonia rather than diffuse alveolar damage or ARDS, in which involvement tends to be diffuse and bilateral. Histologic examination showed a suppurative process with abundant neutrophils and the presence of fungal yeast cells.

The 8 SARS cases (patients 1–8) showed diffuse alveolar

damage[16] with varying degrees of organization. Macrophages were present in the alveolar spaces, and lymphocytes were sparse. Patients 1, 2, and 3 received mechanical ventilation, ribavirin, and corticosteroid therapy. These patients showed acute and organizing phases of diffuse alveolar damage characterized by hyaline membranes and interstitial edema in the former, and interstitial and air space organization in the latter. Atypical pneumocytes, giant cells, and syncytia were noted in the patients who had spent longer periods in the ICU and undergone mechanical ventilation. Although striking, these changes were thought to be within the spectrum of severe diffuse alveolar damage.[13,14]

Patient 5 showed a mixture of acute-phase and organizing-phase diffuse alveolar damage, while patients 4, 6, 7, and 8 showed predominantly the acute phase of diffuse alveolar damage. *Pseudomonas aeroginosa* and methicillin-resistant *Staphylococcus aureus* were identified in 2 of 8 SARS patients, and both of these patients had been treated with corticosteroids. An α-hemolytic *Streptococcus* sp was identified in patient 7, and fungal hyphae suggestive of *Aspergillus* and *Mucor* were observed on light microscopy in patient 5. Neither of these patients received corticosteroid therapy.

In situ hybridization-AT tailing–catalyzed signal amplification staining using the oligonucleotide probe to the nucleocapsid protein region of the SARS coronavirus was positive in 3 of 8 SARS patients. The cytoplasm of occasional cells lining the alveoli and sparse cells within the alveolar lumen gave a positive signal. These were patients 7 and 8, the 2 sudden unexpected deaths, and patient 4, who had a severe and rapidly progressive illness.

Cardiovascular.—Eleven of the 14 autopsies were performed on former hospital inpatients who had spent various amounts of time in the ICU and had received mechanical ventilation. Of the SARS patients, 4 of 8 had pulmonary thromboemboli within the main pulmonary artery or segmental pulmonary arteries identified at autopsy. Three had deep vein thrombosis, which in one patient extended to the paraovarian and parauterine veins. In patient 2, marantic valvular vegetations of approximately 5 to 12 mm in diameter involving the mitral, tricuspid, and aortic valves, along with infarction of the heart, kidneys, and spleen and a 2 × 2-cm softening of the right occipital lobe, were observed. Widespread intravascular fibrin thrombi with infarction of multiple organs were noted in patients 2 and 3.

Heart weights of the patients ranged from 286 to 663 g. Histologic examination of the hearts showed isolated myocardial necrosis in 2 patients, fibrin thrombi within myocardial vessels in 2 patients, and focal perivascular inflammation in 2 patients, but there was no definite histologic evidence of myocarditis. Patient 4 showed significant ischemic cardiac pathology with previous subendocardial infarction and coronary vessel occlusion, suggesting that ischemic cardiac pathology contributed significantly to the cause of death.

Other Organs.—Among the SARS patients, changes in the kidneys and liver reflected the severity of the illness. Acute tubular necrosis was seen in 6 of 8 patients, while one showed end-stage nephrosclerosis, and one showed severe autolytic changes. In the non-SARS cohort, 3 showed hypertensive nephrosclerosis, one of whom also showed diabetic glomerulosclerosis. Two patients, one SARS (patient 2) and one non-SARS (patient 11), showed fibrin thrombi within the kidney, suggesting the presence of disseminated intravascular coagulation.

In the liver, varying degrees of centrilobular necrosis and steatosis and a mild portal inflammatory infiltrate were seen in the SARS patients.

Examination of the lymph nodes and spleen showed lymphoid depletion in the SARS patients. While use of corticosteroids may have been a factor in 3 of the cases, the possibility of an immunosuppressive effect could not be discounted. Hemophagocytosis was seen in 3 cases. Among the non-SARS cohort, white pulp depletion was noted in one patient and erythrophagocytosis in another.

COMMENT

The primary pathologic finding in the SARS autopsy cases of our study was pulmonary diffuse alveolar damage with frequent superimposed bronchopneumonia. Diffuse alveolar damage is a nonspecific histologic reaction[13–15] that can occur for a variety of reasons, including infection, sepsis, uremia, drug toxicity, and collagen vascular disease, as well as in an idiopathic setting in which the term *acute interstitial pneumonia* is appropriate.[17]

During the first few weeks of the SARS outbreak, the diagnosis of SARS infections was made by exclusion.[12] A suspected case was defined by symptoms and epidemiology, while a probable case was defined as a suspected case with radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome or else a suspected case with autopsy findings of respiratory distress syndrome without identifiable cause. Our series of autopsies illustrates the difficulty of rendering a diagnosis of SARS at autopsy, when contact information and clinical history may be lacking, particularly in the setting of a sudden unexpected death.

In the context of emerging infectious diseases, when the clinical syndromes, modes, and probabilities of transmission are yet to be clarified but when immediate quarantine measures[3,5] must be implemented, the implications of a SARS versus a non-SARS diagnosis are great. In our series, this decision was complex, even after a complete autopsy had been performed. Also, all of the patients' lungs receiving gross examination in our series were heavy, with combined weights (except for those of patient 9) of more than 1000 g. For 7 patients, death certification had to be deferred or amended, pending or following the results of histopathology examination or virology tests. In 2 cases, this resulted in a delay of quarantine orders.

Analysis of the progression of disease was an important diagnostic deciding factor. Five of 6 of the SARS patients in our study had patchy ground-glass opacities on admission to the hospital, while for the sixth patient, radiographic abnormalities were documented on the fifth day. In a retrospective analysis of 31 SARS cases seen in the Department of Diagnostic Radiology at the Tan Tock Seng Hospital, 21 of the 31 patients presented with small (involving less than half of one lung zone) ground-glass opacities, while 11 had patchy consolidation, and 2 had both. Ten patients showed a peripheral distribution of ground-glass opacities, 11 showed both peripheral and central opacities, and 6 showed only central changes. Hilar enlargement suggestive of enlarged lymph nodes, interstitial/reticular patterns (patient 9), and dense lobar consolidation (patient 11) were not seen in the initial x-ray films from SARS patients. This is similar to the pattern reported from Hong Kong.[18]

Approximately 21.3% of the patients at the Tan Tock Seng Hospital entered a rapidly progressive pulmonary phase, and of these, 84% met the criteria for acute lung injury or ARDS and required mechanical ventilation. A review of the radiographs of 30 patients in the ICU taken immediately after intubation revealed bilateral symmetric air space shadowing in 80% of them (24 of 30). The shadowing consisted of ground-glass opacities and consolidation. The mortality rate of this group as of May 2, 2003, was 43%.

In this autopsy population, a rising trend in total white blood cell counts and proportion of neutrophils (Table 2) was noted with clinical deterioration to acute lung injury and ARDS.

Histologic examination of the lungs of the 8 SARS patients showed diffuse alveolar damage[16] as the major underlying pathology, ranging from acute, early-phase diffuse alveolar damage with minimal inflammatory infiltrate, as in patient 7, to organizing-phase diffuse alveolar damage with superimposed pneumonia, as in patients 1, 2, and 3. We believe that the cases of our study demonstrate a continuum of changes, with cases 4 through 6 fitting in between these 2 extremes. Patterns of lymphocytic interstitial pneumonia were not seen.

The pattern and morphologic features associated with influenza pneumonia have been described.[19,20] The influenza A virus infection is known to lead to the necrosis of lung epithelial cells[21] and has been demonstrated in bronchial epithelium using in situ hybridization.[22]

While the SARS-associated coronavirus is thought to be new to the human population,[23] the coronavirus infection is known in animals. Reports from investigators working on the porcine reproductive and respiratory syndrome virus have suggested that this virus also has a tropism for macrophages in the lungs and lymphoid tissues[24] and that both infected and uninfected bystander cells undergo apoptosis.[25] A similar mechanism may contribute toward the changes that occur in human infections.

A SARS coronavirus was recently reported in the alveolar epithelial cells and alveolar macrophages of one patient.[26] In our series, 3 patients showed a presence of the virus by in situ hybridization-AT tailing–catalyzed signal amplification. Positive signals for the virus were seen in the 2 sudden unexpected deaths (patients 7 and 8) with acute-phase diffuse alveolar damage as well as in patient 4, whose illness was complicated by diabetes mellitus and end-stage renal failure. The virus was not detected in patients 1, 2, and 3 with the more advanced phases of diffuse alveolar damage and longer periods of illness, nor was it detected in patients 5 and 6. Although this requires further study, it is possible that in SARS coronavirus pneumonia, the initial severity of epithelial injury is related directly to the presence of a virus in the alveolar lining cells and that continuing lung damage is due to poorly controlled immune-mediated processes. In this setting, sudden unexpected death may occur because of a rapid and extensive loss of alveolar cells.

Pulmonary thromboemboli (3 of 3), deep vein thrombosis (2 of 3), intravascular microthrombi (3 of 3), and systemic infarction (1 of 3) were observed in the initial (patients 1–3) autopsies performed. Afterward, surveillance protocols were effected in the Tan Tock Seng Hospital SARS ICU. As of May 2, in the experience of the Tan Tock Seng Hospital SARS ICU, 20.5% had deep vein thrombosis, 11.4%, showed clinical evidence of pulmonary embolism, 15.9% had myocardial infarction, and 4.5% had a cerebrovascular accident. Whether this is related to ARDS and multiorgan pathology in the critically ill,[27–29] therapeutic intervention, or viral-associated damage[21,23] remains to be determined.

The revised World Health Organization definition for SARS,[12] issued on May 1, 2003, was welcome, as it provided for the incorporation of results from laboratory assays for the identification of coronavirus into the definition of SARS cases. We believe that diffuse alveolar damage represents the underlying lung pathology in SARS infections, and we recommend that the Centers for Disease Control and Prevention[30] and World Health Organization[12] case definitions of SARS be modified to specifically mention ''pathologic findings of diffuse alveolar damage'' rather than ''pneumonia or respiratory distress syndrome'' of unknown cause, as currently stated.

CONCLUSIONS

In our series of 14 probable and suspected SARS autopsies, 8 could be confirmed as SARS on the basis of virology data. Diffuse alveolar damage was seen as the basic pathology underlying these cases. It is unfortunate that a term such as *atypical pneumonia* has been used in conjunction with SARS. Although nonspecific and generally taken to denote a nonbacterial lung infection,[31] this term does not reflect the underlying dangers of a viral infection with diffuse alveolar damage, which may progress rapidly to ARDS—an entity well known to clinicians.

The patients of our study also showed a wider range of disease manifestation than has been previously described, with 2 presenting at autopsy as sudden unexpected deaths. This is a worrisome finding that illustrates the difficulty of differentiating an important emerging disease from other causes of sudden cardiovascular death at autopsy.

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References

1. Ksiazek TG, Erdman D, Goldsmith C, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med.* 2003;348:1953–1966.

2. Drosten C, Gunther S, Presier W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med.* 2003;348: 1967–1976.

3. Leo YS, Chen M, Heng BH, et al. Centers for Disease Control and Prevention. Severe acute respiratory syndrome—Singapore 2003. *MMWR.* 2003;52: 405–411.

4. Poutanem SM, Loe DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. *N Engl J Med.* 2003;348:1995–2005.

5. Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. Available at: www/scienceexpress.org/ 23 May 2003/. Accessed May 28, 2003.

6. World Health Organization. Cumulative number of reported probable cases of severe acute respiratory syndrome (SARS), World Health Organization, Communicable Disease Surveillance & Response, update 5 May 2003. Available at: http://www.who.int/csr/sars/country/2003_05_05/en/. Accessed May 7, 2003.

7. Ministry of Health. Annual Statistics Bulletin 2002. Singapore: Health Information Management Branch, Ministry of Health; March 2003:27.

8. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;149:818–824.

9. The Infectious Diseases Act. Part III Section 9. Cap 137, 1999, rev ed. Available at: http://statutes.agc.gov.sg. Accessed May 17,2003.

10. World Health Organization. PCR primers for SARS by WHO network laboratories. Available at: http://www.who.int/csr/sars/primers/en/. Accessed May 7, 2003.

11. Nakajima N, Ionescu P, Sato Y, et al. In situ hybridization AT-tailing with catalyzed signal amplification for sensitive and specific in situ detection of human immunodeficiency virus-1 mRNA in formalin-fixed and paraffin embedded tissues. *Am J Pathol.* 2003;162:381–389.

12. World Health Organization. Case definitions for surveillance of severe

acute respiratory syndrome (SARS). Revised 1 May 2003. Available at: http://www.who.int/csr/sars/casedefinition/en/. Accessed May 7, 2003.

13. Travis WD, Colby TV, Koss MN, Rosado-de-Christenson ML, Muller NL, King TE. *Nonneoplastic Disorders of the Lower Respiratory Tract.* Washington, DC: Armed Forces Institute of Pathology; 2001. *Atlas of Nontumour Pathology*; 1st series, fascicle 2.

14. Nash G, Blennerhasset JB, Pontoppidan H. Pulmonary lesions associated with oxygen therapy and artificial ventilation. *N Engl J Med.* 1967;276:368–374.

15. Blennerhesset JB. Shock lung and diffuse alveolar damage. Pathological and pathogenetic considerations. *Pathology.* 1985;17:239–247.

16. Franks TJ, Chong PY, Chui P, et al. Lung pathology of severe acute respiratory syndrome: a study of eight autopsy cases from Singapore. *Hum Pathol.* 2003;348:743–748.

17. Travis WD, King TE, Bateman ED, et al. ATS/ERS International Multidisciplinary Consensus Classification of idiopathic interstitial pneumonia. *Am J Respir Crit Care Med.* 2002;165:277–304.

18. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med.* 2003;348:1986–1994.

19. Hers JFAPh, Masurel N, Mulder J. Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. *Lancet.* 1958;2:1141–1143.

20. Yeldandi AV, Colby TV. Pathologic features of lung biopsy specimens from influenza pneumonia cases. *Hum Pathol.* 1994;25:47–53.

21. Arndt U, Wennemouth G, Barth P, et al. Release of macrophage migration inhibitory factor and CXCL8/interleukin-8 from lung epithelial cells rendered necrotic by influenza A virus infection. *J Virol.* 2002;76:9298–9306.

22. Guarner J, Shieh WJ, Dawson J, et al. Immunohistochemical and in situ hybridization studies of influenza A virus infection in human lungs. *Am J Clin Pathol.* 2000;114:227–233.

23. Holmes KV. SARS-associated coronavirus. *N Engl J Med.* 2003;20:348:1948–1951.

24. Duan X, Nauwynck HJ, Pensaert MB. Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus. *Vet Microbiol.* 1997;56:9–19.

25. Labarque GG, Nauwynck HJ, Van Reeth K, Pensaert MB. Apoptosis in the lungs of pigs during an infection with a European strain of porcine reproductive and respiratory syndrome virus. In: Lavi E, Weiss SR, Hingley ST, eds. *Advances in Experimental Medicine and Biology. The Nidoviruses.* New York, NY: Kluwer Academic/Plenum Publishers; 2001.

26. Nakajima N, Asahi-Ozaki Y, Nagata N, et al. SARS coronavirus-infected cells in lung detected by new in situ hybridization technique. *Jpn J Infect Dis.* 2003;56:139–141.

27. Montgomery AB, Stager MA, Carrico JC, Hudson LD. Causes of mortality in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis.* 1985;132:485–489.

28. Abraham E. Coagulation abnormalities in acute lung injury and sepsis. *Am J Respir Cell Mol Biol.* 2000;22:401–404.

29. Kolleff MH, Schuster DP. The acute respiratory distress syndrome. *N Engl J Med.* 1995;332:27–37.

30. Centers for Disease Control. Updated interim US case definition of severe acute respiratory syndrome (SARS). May 23, 2003. Available at: http://www.cdc.gov/ncidod/sars/casedefinition.htm. Accessed May 24, 2003.

31. Swartz MN. Approach to the patient with pulmonary infections. In: Fishman AP, Elias JA, Fishman JA, Grippi MA, Kaiser LR, Senior RM, eds. *Fishman's Pulmonary Diseases and Disorders.* 3rd ed. New York, NY: McGraw Hill; 1998.