

Clinical and Laboratory Findings of SARS in Singapore

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Abstract

Introduction: Singapore was one of 29 countries worldwide affected by severe acute respiratory syndrome (SARS) in 2003. **Materials and Methods:** There were 238 cases identified during the outbreak. We performed a retrospective analysis of the clinical and laboratory data of 234 patients admitted to Tan Tock Seng Hospital and Singapore General Hospital. **Results:** The mean age of patients was 21 years, 31.6% of patients were males and 41.8% were healthcare workers. At presentation, the common symptoms were fever, myalgia, cough and headache; rhinorrhoea was uncommon. On admission, 21% had leukopenia, 18% had thrombocytopenia, 29% had hyponatraemia, 31% had hypokalaemia, 21% had transaminitis. Polymerase chain reaction (PCR) testing of respiratory and stool samples provided the best yield at the end of the first week of illness. Thirty-two patients were initially not recognised as probable SARS and were reclassified when the serology test results were available. The chief reasons for not identifying these patients early were persistently normal chest X-rays (68.8%), very mild presentation (43.8%) and the presence of a concomitant illness (12.5%). Overall, 12% of the patients were probable SARS with atypical presentations. Overall mortality was 11.8%. **Conclusion:** Patients infected with the SARS coronavirus had a wide clinical presentation with non-specific symptoms.

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Key words: Atypical, Clinical, Polymerase chain reaction, SARS, Serology

Introduction

An outbreak of atypical pneumonia was recognised in Singapore soon after the release of a global alert by the World Health Organization (WHO) on 12 March 2003. This disease was later named severe acute respiratory syndrome (SARS). A total of 238 cases of SARS were identified in Singapore with 33 deaths. Globally, 8096 cases of SARS were identified in 29 countries, with 774 deaths.¹

The index patient was admitted to Tan Tock Seng Hospital (TTSH) on 1 March 2003 for atypical pneumonia following a trip to Hong Kong on 25 February 2003. Extensive microbiological work-up for known aetiological agents

were unrevealing. Empiric treatment with levofloxacin proved unhelpful. It was later established that while overseas, the patient had stayed in the same hotel as the index case in Hong Kong.²

The disease was subsequently transmitted to other patients, healthcare workers and visiting friends and relatives,³ and was initially limited within the hospital. As the epidemic progressed, the disease eventually reached the community. TTSH was designated the sole hospital for managing SARS in Singapore by the Ministry of Health. All cases diagnosed to have suspect or probable SARS were admitted or transferred here, unless they were deemed medically unstable. Eventually, the SARS outbreak in Singapore was

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declared over on 31 May 2003.

Worldwide, there was an unprecedented rapid exchange of clinical and technical information⁴⁻⁶ as the disease spread across the continents.² The WHO and the Centers for Disease Control and Prevention (CDC) provided management guidelines on the internet, and early online publications offered managing clinicians a glimpse of this unknown disease.⁷⁻¹⁰ As the epidemic progressed, the wide clinical spectrum of the disease became more apparent. SARS was not always catastrophic as reported. It may instead present atypically.¹¹⁻¹³ The effectiveness of the WHO guidelines were questioned.¹⁴⁻¹⁶

On the technical front, there was a proliferation of polymerase chain reaction (PCR) assays.^{8,9,17-20} Molecular detection was attempted in various clinical samples across the duration of the acute illness.²¹⁻²⁵ Initial reports suggest that respiratory samples were most helpful at the initial presentation, followed by stool specimens in the later half.^{21,23}

In this paper, we describe the clinical and laboratory features of 234 probable SARS patients who were admitted to TTSH or Singapore General Hospital (SGH), including 32 patients who were reclassified as probable SARS after the acute illness was over. In addition, results of PCR testing on these probable SARS patients in the Singapore context are presented and compared against other reports.

Materials and Methods

Patients

A total of 206 patients were diagnosed to have probable SARS during their hospital admission, or at autopsy. Another 32 patients were reclassified as probable SARS cases when the serology results were available after discharge. Only patients who were admitted to TTSH or SGH were studied. Demographic, clinical and laboratory data, and outcome measures were collected retrospectively through the review of clinical notes and computerised laboratory results.

Definitions

Suspect case: A case with fever plus one of the following: (1) Close contact with a known case of SARS within 10 days of onset of symptoms, or (2) having visited at-risk locations (hospitals or workplaces where the disease was reported), or (3) history of travel, within 10 days of onset of symptoms, to an area in which there were reported foci of transmission of SARS. This definition differed from the prevailing WHO definition of suspect case²⁶ in that respiratory symptoms were not a requirement.

Probable case: A suspect case with radiographic findings of pneumonia or respiratory distress syndrome, or pathological features at autopsy that were consistent with

SARS, or positive for SARS coronavirus (SARS-CoV) on reverse transcription polymerase chain reaction (RT-PCR) testing in 2 specimens taken from 2 different sites, OR positive serology for SARS-CoV.

Day one of illness: This was defined as the day of onset of fever.

Laboratory Testing

RT-PCR: RT-PCRs were performed at SGH, TTSH, National University Hospital, Defence Medical Research Institute, and Defence Science Organisation. The last 2 are research laboratories. Specimens collected at TTSH were randomly dispatched to the various laboratories. Weekly meetings ensured standardised testing procedures. RT-PCR was performed on various clinical samples. Initial RT-PCR assays, available at the end of March 2003, used the primers SARS1S/As as described in the paper by Drosten et al,⁸ as well as primers Cor1/2 from the Government Virus unit, Hong Kong.¹⁷ A positive result must be confirmed by re-extraction from the original sample and has to be positive on both sets of primers. After 2 May 2003, RT-PCR tests were done using the RealArt HPA SARS-coronavirus RT PCR kit (Artus GmbH, Germany) on the Lightcycler®, a real-time PCR instrument (Roche Molecular Systems, Pleasanton, USA). A positive result was defined as the detection of ≥ 2500 copies/mL of specimen. A negative result was defined as the detection of < 2500 copies/mL. Specimens with positive results were further confirmed by re-extraction from the original sample with a second RT-PCR using primers designed by the Genome Institute of Singapore¹⁸ or the Institute of Molecular and Cell Biology.¹⁹ The latter primers targeted the proteinase gene region, while the rest targeted the polymerase gene of the SARS coronavirus (SARS-CoV). Various specimens were accepted for clinical testing, including respiratory samples (sputum, nasopharyngeal aspirate, endotracheal aspirate, throat swab), urine, conjunctival swab, stool and blood (plain and EDTA-anticoagulated blood).

Serology: Patients were tested for virus-specific IgM, IgG and IgA using an indirect enzyme immunoassay²⁷ with SARS-CoV lysate as the antigen.²⁰ Positive and negative controls were performed concurrently. Detection of fluorescence at a titre ≥ 400 constituted a positive test. Positive sera were re-tested for IgG and IgM by an immunofluorescence assay using SARS-CoV-infected Vero cells spotted onto microscope slides. All serology tests were performed in one centre (SGH).

Statistical Analysis

Data were analysed using the Stata version 6.0 software (Stata Corp, College Station, Texas, USA), and all tests were conducted at the 5% level of significance.

Results

A total of 238 cases of probable SARS were identified in Singapore. Two hundred and six cases were diagnosed as in-patients whilst 32 additional cases were identified after discharge when the serology results became available. Two hundred and thirty-one cases were admitted to TTSH, 3 to SGH, and 3 to 2 other local hospitals. One patient died at home. For the latter, the diagnosis of SARS was based on autopsy findings. Only cases from TTSH and SGH, numbering 234, were analysed.

Laboratory confirmation of probable SARS was available for 214 patients. A rising titre was demonstrated in their serology test results for 213 patients while 1 patient had >1 positive PCR tests in the samples processed. One other patient had autopsy-proven SARS. The other 19 patients were diagnosed purely on clinical grounds in the presence of a strong epidemiological link; 7 had SARS and were established to have transmitted SARS to other individuals; 5 were members of the same household with a patient who had SARS; 5 had come in contact with patients who had SARS either as healthcare workers, visitors, or as fellow patients in the same ward; 1 had had exposure to a patient with SARS at work (not in hospital); 1 had contracted it while overseas. All patients satisfied the probable SARS criteria. The criteria for diagnosis of probable SARS did not differ from the WHO's recommendations.²⁶

The mean age of the patients was 21 years (range, 1.3 to 83.4). Thirty-one (13.0%) patients were aged 21 years and below. Seventy-four (31.6%) were males, and 98 (41.8%) were healthcare workers (14 doctors, 56 nurses and 28 paraclinical staff). Several patients had been exposed to patients with SARS in their course of work as healthcare workers, or as family members. The period of exposure ranged from 1 to 33 days. To provide a more sensible estimation of the incubation period, only patients with exposure to patients with probable SARS of not more than 2 days were included in estimating the incubation period. The median incubation period was thus 5 days (range, 2 to 15). They were admitted to hospital at a median of 3 days after the onset of symptoms.

All patients reported fever as a symptom at presentation, but 28 patients (20%) did not have a documented fever ($\geq 38.0^\circ\text{C}$) till the next day. Of note, for those patients who were admitted within the first 2 days of illness ($n = 88$), 19 patients (21.6%) recorded at least one temperature reading $>40.0^\circ\text{C}$ during their stay, with the highest temperature recorded at 40.5°C . The median duration of fever was 11 days, and the shortest duration recorded was 2 days.

The common symptoms experienced were fever (92.7%), myalgia (62.4%), and cough (38.5%). Diarrhoea occurred in 6.8% of patients. Other reported symptoms include

headache (15.4%), malaise (10.7%), shortness of breath (13.3%), sorethroat (13.3%), nausea and vomiting (11.1%), and anorexia (6.8%). Rhinorrhoea was uncommon (2.1%).

Plots of the temperature, leukocyte, neutrophil, lymphocyte, platelet counts, serum lactate dehydrogenase and albumin levels by day of illness are shown in Figures 1 to 3. On admission, 49 (20.9%) patients had leukopaenia, 42 (17.9%) had thrombocytopenia, 68 (29%) had hyponatraemia ($<134\text{ mmol/L}$), 73 (31.2%) had hypokalaemia ($<3.5\text{ mmol/L}$), and 50 (21.36%) had transaminitis ($\text{ALT} >45\text{ IU/mL}$).

The lowest documented platelet count was $9 \times 10^6/\text{mL}$. This was in a patient who developed disseminated intravascular coagulopathy (DICC). He eventually died on Day 6 of illness. Excluding patients who developed complications of DICC, the lowest platelet count observed was $54 \times 10^6/\text{mL}$ in a patient on Day 8 of illness.

Information on chest radiographs on admission were available for 129 individuals. Of these, abnormal infiltrates were identified in 118 (91.5%) patients; 35 (30%) were bilateral, 83 (70%) were unilateral.

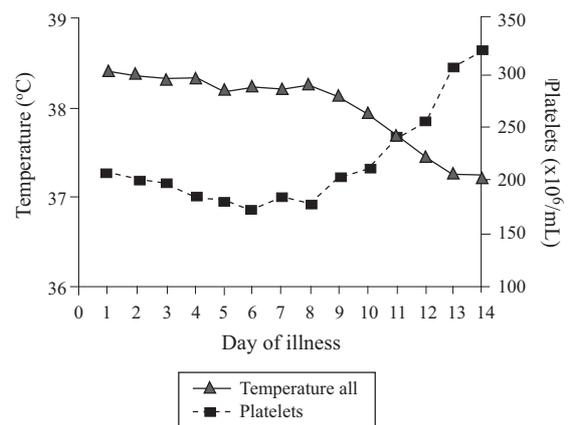


Fig. 1. Temperature/platelets by day of illness.

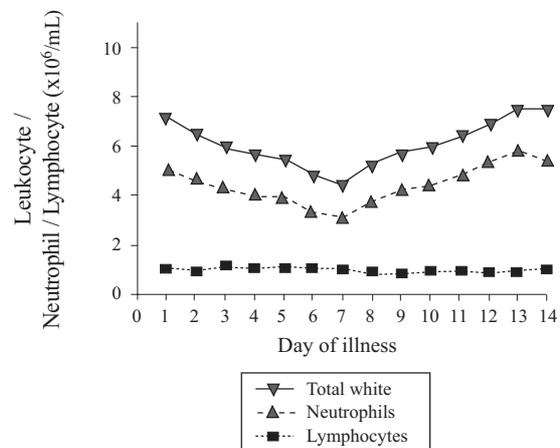


Fig. 2. Leukocyte/neutrophils/lymphocytes by day of illness.

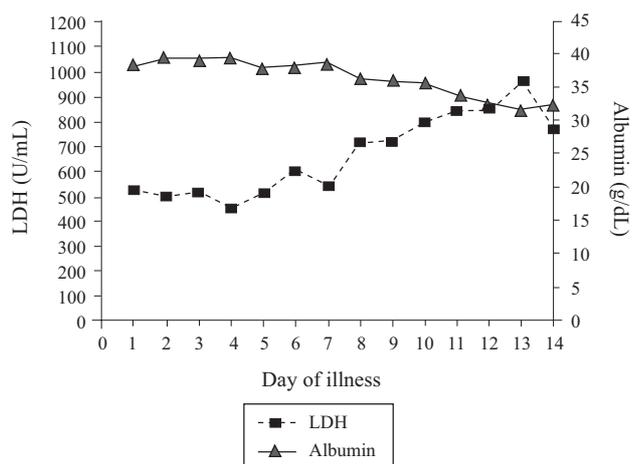


Fig. 3. LDH/albumin by day of illness.

All patients received anti-bacterial agents on admission. Levofloxacin, or a combination of ceftriaxone and a macrolide (erythromycin or clarithromycin) was used. This was in accordance with WHO guidelines.

Ninety-seven (41.4%) patients received ribavirin. There was no apparent clinical benefit.²⁸ Steroids were used in 40 (17.2%) patients in the form of oral prednisolone, intravenous hydrocortisone, pulse methylprednisolone or in combination. Of these, 19 patients (47.5%) died. This was in contrast with 11/194 (5.7%) who died without steroid administration ($P < 0.0001$). Indications for use included treatment for SARS^{29,30} or acute bronchospasm. The data was too skewed for further analysis.

In the entire cohort, 30 (11.8%) patients died. Cause of death was attributed to SARS. A total of 46 (19.7%) patients were admitted to the ICU, of which, 40 received mechanical ventilation. The median length of hospital stay was 12.0 days (range, 1 to 101) for the entire cohort. None of the 12 paediatric patients (age <17 years) was admitted to the ICU or died.

RT-PCR Testing

A total of 203 specimens from 71 probable SARS patients were collected during the first 14 days of illness. Various types of specimens were collected at different time points for analysis; they were plotted against day of illness (Fig. 4). Respiratory samples consisted of nasopharyngeal specimens (6% of total number of specimens), throat swabs (10%), nasal swabs (5%), and sputum samples (6%). The proportion of specimens positive for SARS-CoV (PCR) by specimen type is shown in Figure 5.

The overall diagnostic sensitivity was 33.5% for specimens collected within the first 14 days of illness. Respiratory and stool specimens provided the best yield at 49% and 43%, respectively. Blood, urine and saliva, though

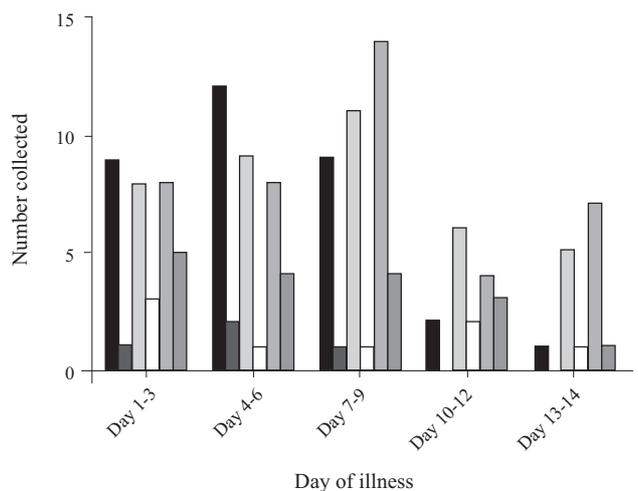


Fig. 4. Specimens collected by day of illness.

very convenient to collect, gave very poor yields – 9.3%, 5.5% and 0% respectively. Three patients had positive PCR results from their tear specimens and were positive in the first 9 days of illness.²⁵ In general, specimens collected towards the end of the first week and at the beginning of the second week had the best yields (Fig. 5).

A total of 310 samples were collected after Day 14 of illness from 111 patients. Similar sample types were collected, namely blood (21), conjunctiva (3), respiratory aspirates or swabs (68), saliva (46), stool (163) and urine (9). Of these, only 7 stool samples, 2 respiratory samples and 1 urine sample tested positive for SARS-CoV. The last positive respiratory and stool samples were collected on Days 33 and 37 of illness.

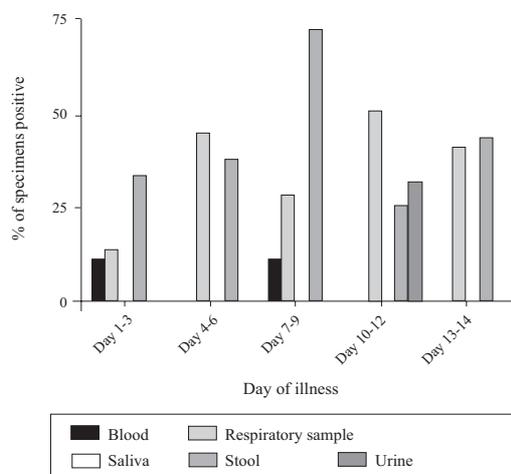


Fig. 5. Percentage of specimens positive on PCR for SARS-CoV by specimen type.

Serology Results

Two hundred and twelve patients submitted their blood for serology testing. The cumulative plot of SARS-CoV serology against day of illness is shown in Figure 6. Four patients had a negative serology result on follow-up. In these individuals, serology tests were performed between 47 and 54 days after the onset of illness. Overall, the centre performed serology testing for 2764 individuals. Four individuals had false positive results. Three individuals had persistently the same titres (400) performed monthly over a 3-month period. The fourth individual had a initial titre 1600 at admission, but titres was 400 in the subsequent 3 months. She presented with a febrile illness in September 2003, months after the SARS epidemic was over. Overall specificity was thus 98.6% and sensitivity was 98.1%.

Reclassification of Cases from Suspect SARS to Probable SARS

Reclassification of cases was done when the serology result returned positive at follow-up. All 32 cases identified had at least a 4-fold rise in serological titres. Six were males. Nine were healthcare workers. Each patient's file was re-examined by one of the authors (NLH) and reasons were identified as to why the diagnosis of probable SARS was not made during the period of the acute illness. The principal reason was the absence of an abnormal chest radiograph in 22 (68.8%) patients. Fourteen (43.8%) had a short duration of fever (<7 days), some having had fever only for 2 days. These patients had mild illness and were atypical compared to the catastrophic cases described earlier.⁷⁻¹⁰ Five (15.6%) patients gave no history of contact, 4 (12.5%) patients had concomitant illness, and 3 (9.4%) patients were unrecognised because they had fallen ill very early in the epidemic, when the disease was still unknown.

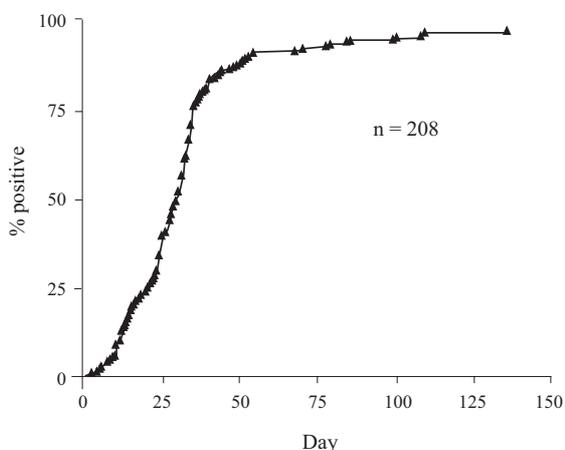


Fig. 6. Cumulative plot of SARS-CoV serology against day after onset of fever.

Fifteen patients had more than one reason identified. Concomitant illnesses identified included acute pulmonary oedema, *Escherichia coli* bacteraemia, urinary tract infection and pre-existing lung disease with an abnormal chest radiograph. Excluding the 3 patients who developed SARS very early in the epidemic, 29 (12.4%) were probable SARS patients with an atypical presentation.

Diagnostic testing with PCR was performed for 6 of these patients prior to serological testing. A total of 13 samples (mean, 2.1; range, 1 to 7) were analysed. It was not offered for the others as they were admitted when the RT-PCR diagnostic kit was not available, or they had very mild disease with normal chest radiograph. Only 2 samples from 2 different patients were positive. Both had normal chest radiographs. Serological testing was positive after the resolution of fever, and hence the criteria for the diagnosis of SARS were only satisfied much later.

Discussion

The definition of suspect SARS in our working criteria during the epidemic differed from the WHO recommendations²⁶ in that respiratory symptoms were not a requirement. Early experience suggested that cough was not a pre-requisite.^{3,31-34} By excluding this requirement, we were able to identify patients who were in the early stages of SARS. Indeed, in this cohort, only 38.5% of patients reported cough, 13.3% had shortness of breath and 42.7% had either or both respiratory symptoms at admission. Cough eventually developed in 55.1% of our patients. Our criteria lowered the specificity in identifying SARS patients, but allowed us to rapidly isolate infected patients and contain the epidemic. Ninety-five (40.6%) patients were admitted within the first 3 days of onset of illness. Otherwise, the diagnosis of probable SARS remained the same as WHO guidelines.

Retrospectively, the sensitivity of WHO guidelines in diagnosing suspected SARS in our cohort of patients was 43% at admission. Others reported a sensitivity of 25% to 28% in the setting of a busy emergency department.^{15,16} The higher figure reported in our study is likely due to the patients having more advanced illness and being more symptomatic at the time of admission. Looking solely at temperature as a criterion, 28 (20%) patients did not have a fever on admission. Fever eventually developed within 24 hours of admission. This "absence of fever" in atypical SARS was similarly reported by Fisher and colleagues,¹¹ and only further confounded the diagnosis.

Overall, the presenting symptoms of this cohort of patients were similar to the Canadian¹⁰ and Hong Kong experience.⁷ Slight variations exist. The former had more patients with cough (69.4%), dyspnoea (41.7%), headache (35.4%) and malaise (31.2%), and fewer complaints of myalgia (49.3%).

In Hong Kong,⁷ cough (57.3%), headache (55.8%) and diarrhoea (19.6%) were more common. Interestingly, rhinorrhoea was infrequent in our study, similar to the Canadian study. In the Hong Kong study, it was reported in 22.5% of the patients.

These symptoms were similar to those of many viral infections, making an initial diagnosis without laboratory or radiological aids difficult. Several patients with probable SARS were wrongly admitted with the diagnosis of dengue fever. With increased awareness of SARS, the converse occurred; patients with dengue fever were admitted as suspect SARS. Of particular interest, 4 patients (2%) with probable SARS demonstrated a saddle back fever during their first week of illness, further mimicking dengue fever. This “return of fever” was found in <10% of patients by Booth and colleagues.¹⁰

Even with the availability of basic laboratory investigations and the chest X-ray, the resemblance to “atypical pneumonia” caused by mycoplasma, legionella or chlamydia was striking. This made it difficult to identify a patient with probable SARS in a non-tertiary hospital. The challenge in identifying a novel infection in the face of a busy emergency department became even more formidable.³⁵ Several alternative diagnoses were eventually found for patients not diagnosed with SARS. This included pulmonary tuberculosis, malignant fever, infective endocarditis, and acquired immunodeficiency syndrome presenting with *Pneumocystis carinii* pneumonia. On the other hand, concomitant bacterial,^{12,36} fungal³⁶ and mycobacterial³⁷ infections with SARS have been reported. This serves to remind clinicians that guidelines are never all-encompassing.

The 32 cases of patients requiring reclassification illustrate the wide clinical spectrum of SARS. Asymptomatic to mild or atypical infections have been described.^{11,38-41} In our series, 12% of the patients had probable SARS but with atypical presentations. Overall, 57.3% presented without respiratory symptoms. Chest radiographs were normal during the acute illness in 22 patients (9.5%). Of note, 1 patient had an abnormal chest radiograph only at Day 16 of illness, after the fever defervescenced on Day 11 of illness. A computed tomography scan of the thorax would increase sensitivity but would be impractical, incur further costs, further stretch limited resources and unnecessarily expose other healthcare workers to the disease. PCR is an expensive, technically difficult test to perform and is not readily available everywhere. Serological testing may become positive only at week 6 to 7 of illness,¹⁷ making identification of SARS during the acute illness difficult. Strict diagnostic criteria such as these may not be applicable in a country with known local transmission.⁴²

RT-PCR testing provided overall yields of 33.5%. This differed from data obtained by other authors from Hong Kong. Peiris and colleagues²¹ reported that 32% of their nasopharyngeal aspirate samples were positive on presentation and 68% were positive at Day 14, while stool samples were 97% positive at Day 14. Tsui and colleagues²² reported overall detection rates of 58%. Chan and colleagues²³ found an overall detection rate of 60%. Ng and colleagues²⁴ performed RT-PCR on 12 plasma samples using primers directed against the polymerase gene. Detection rate was reported as 50% taken at day one of admission. Using sera from a separate batch of 23 samples, the detection rate was 78%. These sera were again tested using other primers directed at the nucleocapsid gene. A higher sensitivity of 87% was achieved for the same set of 23 samples. The better detection rate observed may have been due to the choice of primers at the nucleocapsid gene.

The difference observed may be explained by a few reasons. Firstly, the majority of our patients had their onset of illness in April 2003, when the PCR assays used were being optimised and were not as sensitive as the real-time assays used from May 2003. Secondly, the median number of samples collected from our patients during the course of their illness was just 2. It has subsequently been shown that testing of multiple specimens, especially respiratory specimens, increases the sensitivity of RT-PCR.

Thirdly, many of our initial respiratory samples came highly diluted in Hank's transport media, which would have affected the sensitivity of the PCR assays. Lastly but probably most significantly, it has been reported that steroid use increases plasma viral loads.⁴³ For the Hong Kong papers, all the patients in Peiris's study²¹ and 99.3% of patients in Tsui's study²² were given steroids. In contrast, the majority (83%) of patients in our study did not receive steroids and this may have given rise to lower viral loads in clinical samples and the apparent poorer sensitivity of the PCR assays.

Four patients had negative serological test results at follow-up. These patients were diagnosed clinically to have probable SARS based on existing guidelines. None had SARS viral ribonucleic acid detected. In retrospect, 1 patient probably had dengue fever instead. A rising titre of dengue antibodies was detected. The other 3 patients may simply have had late seroconversions.¹⁷

The overall crude mortality rate was 11.8%. This was comparable to the worldwide crude mortality rate of 9.5%.¹ The paediatric group, though small (n = 12), had neither deaths nor required ICU care (Fisher's exact test $P = 0.23$). This trend towards better prognosis is in concordance with other reports.⁴⁴⁻⁴⁶

Conclusion

SARS-CoV is a pathogen with a wide spectrum of clinical presentations. A large proportion may not present with respiratory symptoms, especially in the early stages. PCR and serology remain the main diagnostic tools in the acute phase, with respiratory and stool samples providing the best yield within the first 2 weeks of illness.

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