Interpretation of diagnostic laboratory tests for severe acute respiratory syndrome: the Toronto experience

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Fast-tracked article. Early release, published at www.cmaj.ca on Dec. 2, 2003. Subject to revision.

Abstract

Background: An outbreak of severe acute respiratory syndrome (SARS) began in Canada in February 2003. The initial diagnosis of SARS was based on clinical and epidemiological criteria. During the outbreak, molecular and serologic tests for the SARS-associated coronavirus (SARS-CoV) became available. However, without a "gold standard," it was impossible to determine the usefulness of these tests. We describe how these tests were used during the first phase of the SARS outbreak in Toronto and offer some recommendations that may be useful if SARS returns.

Methods: We examined the results of all diagnostic laboratory tests used in 117 patients admitted to hospitals in Toronto who met the Health Canada criteria for suspect or probable SARS. Focusing on tests for SARS-CoV, we attempted to determine the optimal specimen types and timing of specimen collection.

Results: Diagnostic test results for SARS-CoV were available for 110 of the 117 patients. SARS-CoV was detected by means of reverse-transcriptase polymerase chain reaction (RT-PCR) in at least one specimen in 59 (54.1%) of 109 patients. Serologic test results of convalescent samples were positive in 50 (96.2%) of 52 patients for whom paired serum samples were collected during the acute and convalescent phases of the illness. Of the 110 patients, 78 (70.9%) had specimens that tested positive by means of RT-PCR, serologic testing or both methods. The proportion of RT-PCR test results that were positive was similar between patients who met the criteria for suspect SARS (50.8%, 95% confidence interval [CI] 38.4%–63.2%) and those who met the criteria for probable SARS (58.0%, 95% CI 44.2%–70.7%). SARS-CoV was detected in nasopharyngeal swabs in 33 (32.4%) of 102 patients, in stool specimens in 19 (63.3%) of 30 patients, and in specimens from the lower respiratory tract in 10 (58.8%) of 17 patients.

Interpretation: These findings suggest that the rapid diagnostic tests in use at the time of the initial outbreak lack sufficient sensitivity to be used clinically to rule out SARS. As tests for SARS-CoV continue to be optimized, evaluation of the clinical presentation and elucidation of a contact history must remain the cornerstone of SARS diagnosis. In patients with SARS, specimens taken from the lower respiratory tract and stool samples test positive by means of RT-PCR more often than do samples taken from other areas.

CMAJ 2004;170(1)

In February 2003 the worldwide outbreak of severe acute respiratory syndrome (SARS) reached Toronto. This first phase of the outbreak, lasting from March to May 2003, resulted in 257 probable and suspect cases and 27 deaths. Shortly after apparent containment of this outbreak, a second phase occurred, from May to June 2003, with an additional 119 probable and suspect cases and 17 deaths.[1]

Early in the outbreak, the search for the etiologic agent of SARS revealed a new coronavirus.[2–4] Laboratory investigations in Canada added evidence that SARS cases in this country were associated with this new coronavirus.[5] The rapid sequencing of the SARS-associated coronavirus (SARS-CoV) genome by 2 independent research groups enhanced the development of new molecular assays by various laboratories around the world.[6,7] Reverse-transcriptase polymerase chain reaction (RT-PCR) tests, primarily targeting the polymerase gene of the virus, were developed, along with serologic tests for antibodies against the newly discovered SARS-CoV. Although these tests were made available during the outbreak, their sensitivity and specificity were unknown because there were no "gold standard" laboratory or clinical definitions for the diagnosis of SARS. The formal disease definitions developed by the World Health Organization (WHO), the US Centers for Disease

Control and Prevention, and Health Canada were necessarily broadly inclusive and nonspecific.[8–10]

Three observational papers describing the Toronto SARS outbreak have been published.[5,10,11] Clinical and epidemiological findings have also been described.[5,10–14] During the outbreak, the optimal specimen type, the proper transport and handling of specimens, and the timing of collection were unknown because the clinical course of SARS was being elucidated at the same time as laboratory tests were being developed.

Our study summarizes the application of first-generation nucleic acid amplification tests and serologic assays during the first phase of the SARS outbreak in Toronto. Despite the lack of a systematic or uniform protocol for collecting specimens for SARS-CoV detection at the affected hospitals in Toronto and the lack of a "gold standard" with which to evaluate these tests, the results in this study illustrate important trends and observations that will be useful for dealing with future outbreaks of SARS.

Methods

Seven hospitals, including 4 academic teaching centres, were included in this study based on their participation in the Ontario Laboratory Working Group for the Rapid Diagnosis of Emerging Infections and their ability to provide relevant clinical information. From these 7 sites, we retrospectively identified 117 patients admitted to hospital who met the Health Canada criteria for suspect or probable SARS. These patients accounted for the majority of SARS patients in hospital with defined clinical, epidemiological and microbiological data in the first phase of the Toronto outbreak. In brief, suspect cases were defined by Health Canada as people who had a fever, respiratory signs and symptoms, and an appropriate travel or contact history in the absence of an alternative diagnosis.[10] During the first phase of the outbreak, probable cases were defined by Health Canada as people who met the suspect case definition and had severe progressive respiratory illness with or without abnormalities on chest radiographs. Although the Health Canada and WHO definitions of suspect cases were identical, WHO defined probable cases as suspect cases that had radiographic changes consistent with SARS.[8] Each patient was classified as either a suspect or probable case after evaluation by the regional public health unit using the Health Canada definitions. Laboratory results were not readily available to clinicians or public health officials at the time of these assessments.

Demographic data, including age, sex, disease onset, dates of hospital admission and outcome, were collected through retrospective chart reviews. A standardized questionnaire was used to abstract the data. All microbiological reports for patients thought to have suspect or probable SARS were extracted and reviewed. In addition to the patient charts, data were collected from the reports from each hospital laboratory, the Central Public Health Laboratory in Toronto and the National Microbiology Laboratory in Winnipeg. Laboratory databases were also searched to ensure capture of all microbiological tests ordered from the time of disease onset to August 2003 (up to 5 months after disease onset). The data were collated and verified by the lead authors (P.T. and M.L.) in conjunction with the individual clinicians at each participating hospital.

Specimens were collected from the SARS patients according to each institution's protocols. In general, nasopharyngeal swabs, throat swabs and blood samples were collected for diagnostic testing from the majority of patients. Specimens from mucosal surfaces (i.e., the nasopharynx, oropharynx [throat] and conjunctiva) were collected with Dacron swabs and placed into sterile containers with 2 mL of viral transport medium. Stool, urine and lower respiratory tract specimens (sputum, endotracheal tube aspirates and bronchoalveolar lavage fluid) were collected into sterile containers. Collection of blood specimens followed standard protocols. Other types of routine specimens obtained to rule out other potential causes of atypical pneumonia were collected depending on the individual hospital's protocol or the clinical circumstances.

Diagnostic testing for SARS-CoV and other agents compatible with atypical pneumonia was done at the National Microbiology Laboratory and the Central Public Health Laboratory as well as at the microbiology laboratories at the Sunnybrook and Women's College Health Sciences Centre and The Hospital for Sick Children in Toronto and St. Joseph's Hospital in Hamilton, Ont. RT-PCR for the SARS-CoV was performed with different sets of primers at the 5 laboratories (an appendix showing the primers appears at the end of this article). In a separate study, we were unable to show significant differences in SARS-CoV detection among these RT-PCR protocols (J. Mahony et al: unpublished data).

Viral culture and serologic tests for SARS-CoV were done at the National Microbiology Laboratory. SARS-CoV was initially isolated on Vero E6 cell cultures in a biosafety level 3 laboratory, but this procedure was subsequently stopped owing to the high volume of specimens and safety concerns. Serologic status was determined by means of both enzyme-linked immunosorbent assay (ELISA) and immunofluorescent assay (IFA) to detect SARS-CoV antibodies. Serologic test results were considered negative if a convalescent sample drawn 28 days or more after the onset of symptoms yielded a negative result and positive if at least one method detected IgM or IgG antibodies at predefined dilutions.[9] In brief, antigen used to coat the ELISA plates was prepared from Vero E6 cells infected with SARS-CoV. Cells were lysed with 1% Triton X-100 in borate buffer (pH 9.0) and sonication. A control lysate was prepared from uninfected Vero E6 cells. Each specimen was screened at a dilution of 1:400 in a well coated with SARS-infected cell lysate and in a well coated with the control (uninfected) cell lysate. Positive specimens were titrated in 4-fold dilutions, starting with 1:100, to determine the antibody titre. For IFA, slides were also prepared using SARS-infected Vero E6 cells. Cells were scraped from the tissue culture flasks and washed in phosphate-buffered saline. Infected cells were mixed with uninfected cells at a ratio of 2:1, and the mixed suspension was spotted onto slides. Slides were gamma-irradiated before fixing with acetone at –20°C. Serum samples were screened by IFA at a dilution of 1:50. Positive samples were titrated starting at 1:25 in 2-fold dilutions.

Routine microbiological tests were also performed according to each hospital's protocols. All blood and urine specimens were cultured for bacteria. When requested, lower respiratory tract specimens were cultured for bacteria and fungi. Urine samples were tested for *Legionella pneumophila* antigen by means of ELISA. Nasopharyngeal swabs were assessed for the common respiratory viruses (influenza A and B; parainfluenza 1, 2 and 3; respiratory syncytial virus; and adenovirus) by means of culture and direct fluorescent antibody (DFA) tests at each institution's laboratory or at the Central Public Health Laboratory. Culture and DFA were also used to detect *L. pneumophila* in respiratory tract specimens. Detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* was

done by means of PCR, serologic testing (enzyme immunoassay for *M. pneumoniae* and IFA for *C. pneumoniae*) or both at the Central Public Health Laboratory. Serologic testing for influenza was done by means of complement fixation, and for *L. pneumophila* by means of IFA, at the Central Public Health Laboratory.

For analysis, diagnostic test results for SARS-CoV and other pathogens were described along with basic demographic and clinical data for each patient. We evaluated the results of the serologic and RT-PCR tests for SARS-CoV in terms of the timing of these tests during the course of illness as well as the detection rates by type of specimen. We also examined the relation between the SARS test results and disease severity as measured by the case definitions (suspect v. probable) and clinical outcome (death v. recovery).

When comparing samples from the same patient (i.e., stool and nasopharyngeal specimens), we used the McNemar test. Confidence intervals were exact and calculated based on the assumption of a binomial distribution.[15]

The research ethics board of each participating hospital and the University of Toronto approved this study.

Results

Of the 117 patients admitted to the participating hospitals, 56 had probable and 61 had suspect SARS according to the Health Canada criteria at the time of the outbreak. As of Oct. 31, 2003, 17 (14.5%) of these patients had died, 16 of whom had probable SARS. There were 74 women (63.2%) in the group. The mean age was 47 (range 17–99) years.

Blood, urine and sputum specimens from all 117 patients were sent for routine bacteriologic culture, but none yielded any pathogenic organisms. Nasopharyngeal swabs were collected from 102 patients for viral culture and DFA tests for the common respiratory viruses; all results were negative. Urine specimens were negative for *L. pneumophila* antigen in all of 12 patients tested, and culture and DFA tests did not yield *L. pneumophila* in all of 8 patients tested. PCR results were negative for *M. pneumoniae* and *C. pneumoniae* in all of 18 patients tested. The results of testing for metapneumovirus were not included in this study, because these data require further evaluation for proper interpretation.

Acute serologic testing was also done for *L. pneumophila*, *M. pneumoniae*, *C. pneumoniae*, and influenza A and B in a small number of patients. Acute infection with *M. pneumoniae* was suggested by positive IgM serologic results in 4 of 14 patients tested. Of the 17 patients tested for *C. pneumoniae* antibodies, 8 patients showed evidence of acute or recent infection based on an IgM titre of at least 1:10 detected by means of IFA. No patient had IgM antibodies to *L. pneumophila* (21 tested) or to influenza A and B (7 tested). These preliminary results do not fully address the possibility of coinfection in SARS with another pathogen. The serologic results are not conclusive because concomitant collection of specimens for testing by culture, antigen detection or PCR to confirm the serologic findings was not done in all cases. In addition, the possibility of passive acquisition of these antibodies from blood transfusion has not been addressed in our study.

Diagnostic test results for SARS-CoV were available for 110 of the 117 patients (Fig. 1). Of the 110 patients, 78 (70.9%) had specimens that tested positive by means of RT-PCR, serologic testing or both methods. A total of 528 specimens were received from 109 patients for RT-PCR testing (Table 1); 59 (54.1%) of these patients had specimens that tested positive. A similar proportion of suspect and probable cases tested positive for SARS-CoV by means of RT-PCR: 30 (50.8%) of 59 suspect cases (95% confidence interval [CI] 38.4%–63.2%), and 29 (58.0%) of 50 probable cases (95% CI 44.2%–70.7%). The Sunnybrook and Women's College Health Sciences Centre, where the majority of patients were admitted, had the highest proportion of RT-PCR positive results (28 [68.3%] of 41 patients).

Overall, the first set of specimens collected from each patient for SARS investigation was positive in 47 (43.1%) of 109 patients. The mean time from onset of illness to collection of the first diagnostic specimens was 3.9 days (standard deviation [SD] 3.8 days; range 0–20 days). For 48 patients who had specimens taken within the first 2 days after illness onset, 17 (35.4%) had at least one specimen that was positive for SARS-CoV as detected by RT-PCR. The rate of SARS-CoV detection by RT-PCR was the highest between days 9 and 11 after illness onset, followed by a gradual reduction over the subsequent 2 weeks (Fig. 2).

When specimens were classified by type, we found that stool and lower respiratory tract specimens had the highest proportion of positive RT-PCR results (Table 1). Lower respiratory tract specimens were positive in 10 (58.8%) of 17 patients (95% CI 35.7%–78.5%). Bronchoalveolar lavage was performed in 6 probable cases on days 5–15 after illness onset; in all 6, the fluid samples were positive for SARS-CoV as detected by RT-PCR. Endotracheal tube aspirates were only positive in 1 of 6 probable cases; the positive aspirate was collected on day 9 of illness and the negative samples on day 5 and on days 17–44. Sputum was positive for SARS-CoV in 5 of 7 patients (4 of 4 probable cases, 1 of 3 suspect cases); the positive sputum specimens were collected on days 3–9 of illness and the negative specimens on days 18–26. SARS-CoV was detected in stool specimens from 19 (63.3%) of 30 patients (95% CI 45.4%–78.2%); the positive stool specimens were collected on days 1–31 of illness. In the 29 cases (14 probable and 15 suspect) in which both stool samples and nasopharyngeal swabs were collected, the detection of SARS-CoV was significantly better in the stool samples ($p = 0.027$) (Table 2).

The majority of specimens received were nasopharyngeal and throat swabs (Table 1). Nasopharyngeal swabs were positive for SARS-CoV in 33 (32.4%) of 102 cases (95% CI 24.1%–42.0%), and throat swabs were positive in 22 (36.1%) of 61 cases (95% CI 25.2%–48.7%). Positive nasopharyngeal swabs and throat swabs were collected

within the first 17 and 15 days of illness respectively. Of 102 patients who had at least one upper respiratory tract specimen (nasopharyngeal or throat swab) collected, the specimen was positive in 44 (43.1%; 95% CI 33.9%–52.8%). In the 60 cases in which both nasopharyngeal and throat swabs were collected, a higher number of throat swabs than of nasopharyngeal swabs were positive; however, this difference did not reach statistical significance ($p = 0.06$) (Table 2).

A large number of whole blood specimens were also

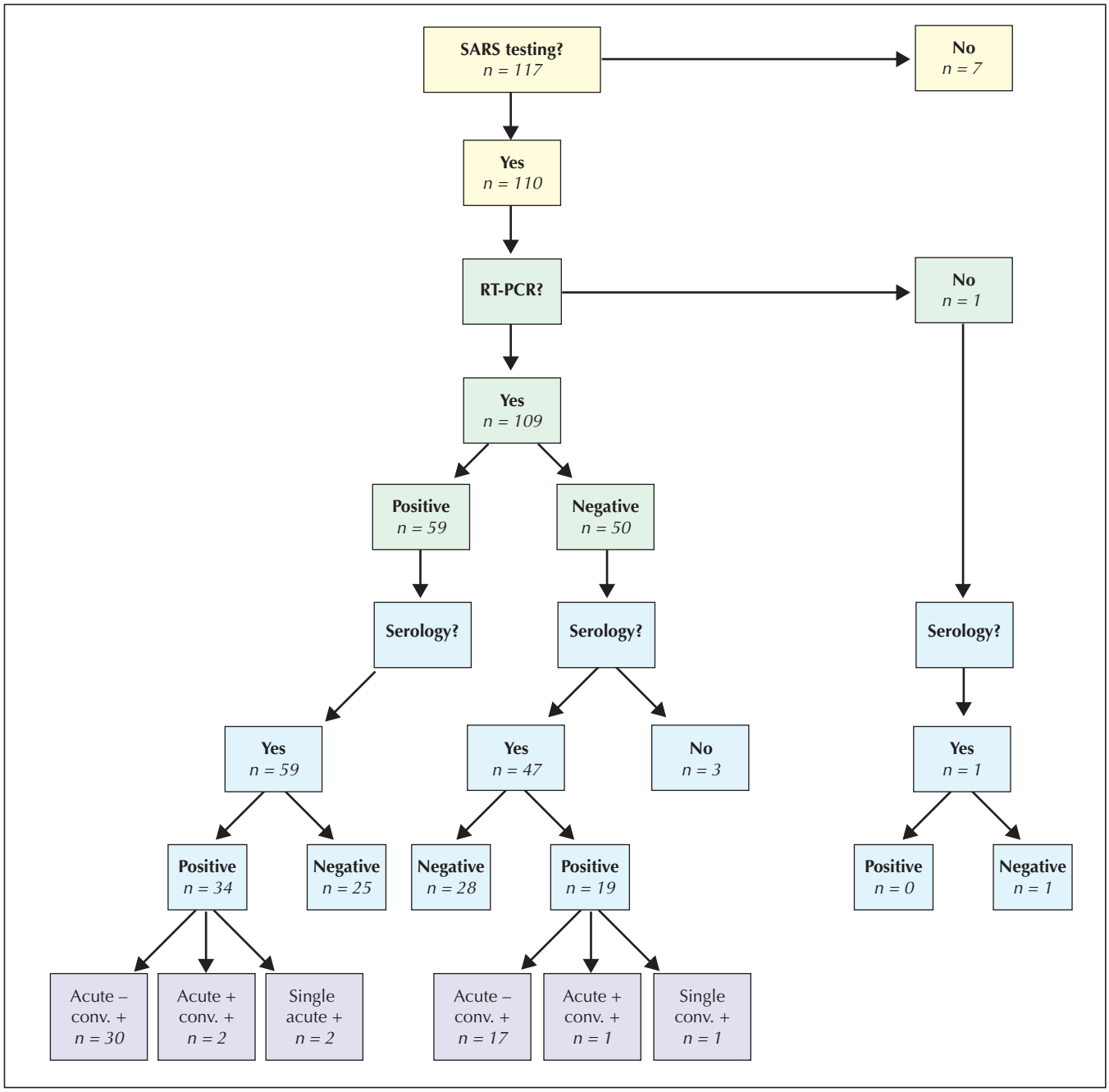

Fig. 1: Diagnostic testing for severe acute respiratory syndrome coronavirus (SARS-CoV) in patients admitted to hospital in Toronto who met the Health Canada criteria for suspect or probable SARS. A total of 110 patients had specimens tested by means of reverse-transcriptase polymerase chain reaction (RT-PCR) ($n = 109$) or serologic testing ($n = 107$). Of the specimens tested by RT-PCR, 59 (54.1%) were positive. Of the 110 patients whose specimens underwent RT-PCR or serologic testing, 78 (70.9%) had specimens that tested positive by one or both methods. A similar proportion of suspect and probable cases had positive RT-PCR results (50.8% and 58.0% respectively). Of the 52 patients with paired serum samples, 50 (96.2%) were seropositive against SARS-CoV; 32 (64.0%) of these 50 patients also had positive RT-PCR results.

received, but the specimens were positive in only 2 (2.5%) of 81 cases (95% CI 0.7%–8.5%). The positive blood specimens from these 2 patients were collected on days 3 and 9 of illness. Both had probable SARS with severe respiratory illness, and one eventually died. One female patient had a urine specimen collected on day 21 that was positive for SARS-CoV, but stool specimens collected on days 12 and 21 were also positive, which suggests possible contamination of the perineal region. Three patients had SARS-CoV detected only from their conjunctival swabs that were collected on days 0, 1 and 4 of illness. Only 1 of these patients had positive convalescent serologic test results; the other 2 did not have convalescent sera collected to support the clinical diagnosis. The significance of SARS-CoV detected in the eye during the natural course of illness is unclear. A better understanding of the pathogenesis of SARS-CoV will be needed to address these findings.

A total of 262 serum samples were obtained from 107 patients for serologic testing for SARS-CoV antibodies. The results of IFA and ELISA were in agreement for all but one of the samples, for which only the ELISA result was positive. This one sample with discordant results was collected on day 22 of illness from a person with suspect SARS who was seronegative on days 1 and 6. A small proportion (8.3%) of the 107 patients were found to be seropositive in the first 2 weeks of illness (Fig. 3). The proportion who were seropositive markedly increased between days 14 and 27of illness.

For 52 of the 107 patients, paired acute and convalescent serum samples were collected. Of these 52 patients, 50 (96.2%) were found to be seropositive against SARS-CoV; 32 (64.0%) of the 50 also had positive RT-PCR results (Fig. 1). For 47 of the 50 patients, the acute sample was negative and the convalescent sample was positive; for the other 3 patients, both the acute (collected on days 8, 10 and 27 of illness) and convalescent samples were positive. For 2 of the 47 patients with negative acute samples, the acute samples were collected on days 0 and 2 of illness, and seroconversion was documented on days 1 and 5 respectively. Of the 52 patients with paired serum samples, the seropositivity rate was similar among the 25 with probable SARS (96.0%, 95% CI 80.4%–99.1%) and the 27 with suspect SARS (96.2%, 95% CI 81.7%–99.1%).

For 2 of the 52 patients with paired serum samples, both the acute and convalescent samples were seronegative; these 2 patients also had negative RT-PCR results. One of these patients had suspect SARS with less severe illness, and the other had probable SARS and was the only patient in the group not epidemiologically linked to the Toronto index case. Possible explanations for the negative serology results in these 2 cases include an inability to mount a detectable humoral response against SARS-CoV, a delayed humoral response or a lack of infection with SARS-CoV despite meeting the SARS case definition.

Fifty-four patients had only an acute serum sample col-

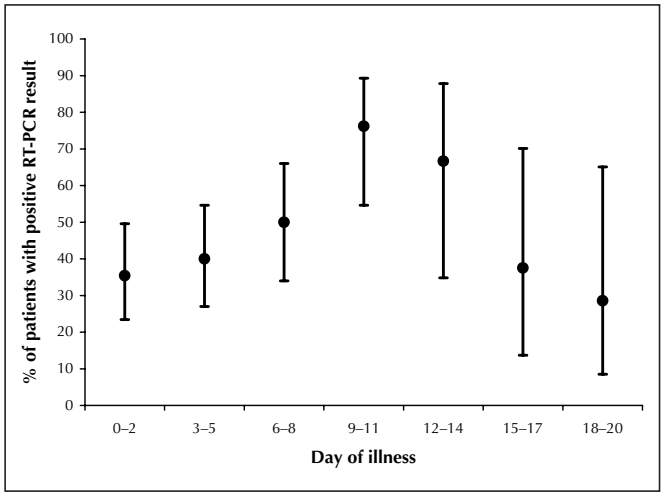

Fig. 2: Proportion of patients with RT-PCR results positive for SARS-CoV, by day of illness. The results are shown for all specimen types. Error bars represent 95% confidence intervals.

Table 1: Detection of severe acute respiratory syndrome coronavirus (SARS-CoV) by reverse-transcriptase polymerase chain reaction (RT-PCR) in patients admitted to hospitals in Toronto

Specimen type	No. (and %) of specimens that tested positive	No. (and %) of SARS cases with positive specimens		
		Suspect cases	Probable cases	Total
Nasopharyngeal swab	53/189 (28.0)	14/56 (25.0)	19/46 (41.3)	33/102 (32.4)
Throat swab	26/78 (33.3)	11/38 (28.9)	11/23 (47.8)	22/61 (36.1)
Stool specimen	26/50 (52.0)	9/15 (60.0)	10/15 (66.7)	19/30 (63.3)
Lower respiratory tract specimen*	21/37 (56.8)	1/3 (33.3)	9/14 (64.3)	10/17 (58.8)
Conjunctival swab	3/26 (11.5)	3/14 (21.4)	0/7 (0.0)	3/21 (14.3)
Whole blood	2/113 (1.8)	0/39 (0.0)	2/42 (4.8)	2/81 (2.5)
Urine	2/35 (5.7)	0/11 (0.0)	1/9 (11.1)	1/20 (5.0)
All	133/528 (25.2)	30/59 (50.8)	29/50 (58.0)	59/109 (54.1)

Note: EDTA = ethylenediaminetetraacetic acid.
*Sputum, endotracheal tube aspirates or bronchoalveolar lavage fluid.

lected for serologic testing; in 2 cases the sample was already seropositive on day 15 of illness. Another patient had only a convalescent sample collected (on day 34 of illness), which was found to be seropositive. Of the 54 patients with only acute serology results, all but 1 of the 52 found to be seronegative had blood drawn within the first 17 days of illness; the other patient had blood drawn on day 26.

Among the 50 seropositive patients with paired samples, the same types of specimens as those in the total group tested by means of RT-PCR were most likely to be positive: stool samples (68.1%, 95% CI 47.1%–83.6%) and lower respiratory tract specimens (80.0%, 95% CI 48.2%–94.0%).

Of the 17 patients who died, 15 had pre-mortem specimens submitted for RT-PCR testing. For 10 (66.7%) of these patients, the test result was positive for SARS-CoV in the first set of specimens collected (nasopharyngeal or throat swabs in 7, bronchoalveolar lavage fluid in 2, and sputum in 1). The mean time from illness onset to presentation was 5.1 (SD 5.0) days (range 0–15 days). Only 3 patients had paired acute and convalescent serum samples collected before death, and all patients seroconverted. Postmortem lung tissue was obtained from 9 of the patients who died; all 9 were found to be positive for SARS-CoV by means of RT-PCR.[16] When pre- and postmortem RT-PCR results were combined, 14 (93.3%) of the 15 patients were found to be positive for SARS-CoV.

Interpretation

Although the WHO has previously posted guidelines for specimen collection for SARS, our study addresses the diagnostic yield of the various specimen types.[17] We found that 70.9% of our SARS patients had at least one specimen that was positive for SARS-CoV, whereas 29.1% had specimens that were negative by all tests. Assuming that all of the patients truly had SARS, the diagnostic sensitivity of combined RT-PCR and serologic testing would be only 70.9% and RT-PCR alone 54.1%. Stool and sputum specimens show promise as the best and least invasive specimen types for RT-PCR. Higher viral loads in the gastrointestinal and lower respiratory tracts may account for the better detection of SARS-CoV in these specimens. The lower proportion of positive RT-PCR results from nasopharyngeal and throat swabs suggests that SARS-CoV does not replicate or reside in the upper respiratory tract at concentrations similar to those of other common respiratory viruses, where their presence is detectable using much less sensitive detection methods (e.g., DFA). Other factors contributing to the low overall sensitivity of detection might also include differences in optimization of RNA extraction and RT-PCR methodologies, deterioration of clinical specimens during transport and handling, and delays in processing specimens. In addition, the peak positivity rate of RT-PCR occurred late, at days 9 to 11 of illness, making RT-PCR less useful during the first week of illness. These findings are similar to those reported by Peiris and colleagues[18] for the outbreak at Amoy Gardens housing estate in Hong Kong.

Serologic testing appeared to be the best method for confirming SARS cases in our study: the seropositivity rate reached 96.2%. Serologic analyses of SARS patients in Hong Kong showed similar rates (93%–99%).[18,19] However, convalescent serum samples were not collected from all patients in our study, especially those who had mild illness and did not return for follow-up; therefore, sampling bias may have contributed to the high proportion of patients who tested positive late in the disease course. Also, 2 patients appeared to have seroconverted within the first week of illness; this may represent an inaccuracy in the date of illness onset, which was based on information provided by the patient rather than on true seroconversion in the first

Table 2: RT-PCR detection of SARS-CoV in nasopharyngeal swabs compared with detection in stool specimens or throat swabs among patients with paired specimens

	Nasopharyngeal swab; no. of patients		
Specimen; result	Positive	Negative	Total
Stool specimen			
Positive	8	11	19
Negative	2	8	10
Total	10	19	29
$p = 0.027$			
Throat swab			
Positive	11	11	22
Negative	3	35	38
Total	14	46	60
$p = 0.06$			

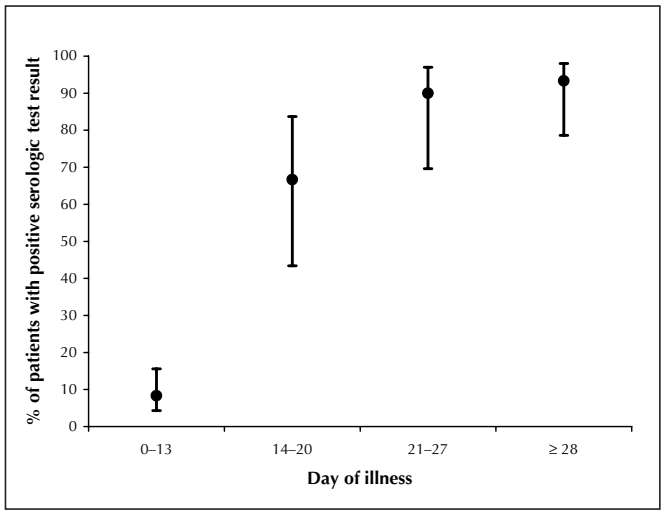

Fig. 3: Proportion of patients with serologic test results positive for SARS-CoV antibodies, by day of illness. Error bars represent 95% confidence intervals.

week of illness. Even if some patients seroconverted early, the utility of serologic diagnosis is confined to retrospective analysis owing to the generally long lag time to seroconversion. Nonetheless, serologic testing of convalescent samples should still be done for all patients in whom the diagnosis of SARS is entertained.

Our study was limited by the use of retrospective data, the inability to test sequential specimens collected at defined times during the course of illness in all cases, and the lack of standardized specimen collection and testing protocols. For example, at the midpoint of the first phase of the outbreak, nationwide directives were issued that temporarily prohibited the collection of nasopharyngeal swabs because they were thought to cause aerosolization of the virus and thus an unnecessary risk to health care workers. The observations in our study require further validation in controlled, prospective studies. In the interim, we suggest that stool and multiple respiratory tract specimens from patients with suspect or probable SARS be sent for RT-PCR testing for SARS-CoV. A negative test result would not rule out SARS, but a positive result, either with or without an epidemiological link, would warrant further infection control precautions and investigation, including repeat diagnostic testing for SARS. The tests used in this study were not useful for ruling out disease in the individual patient; however, they are important for diagnosing clusters of patients who present with similar signs and symptoms. Still, clinical judgement and the identification of an epidemiological link remain crucial when one is faced with a potential SARS case during an outbreak. However, for sporadic cases with no epidemiological links and where prevalence rates are very low, assays with increased sensitivity and careful interpretation of any results will be required. The Ontario Laboratory Working Group for the Rapid Diagnosis of Emerging Infections is currently conducting more definitive studies using improved assays on a well-defined cohort of SARS cases to evaluate the impact of specimen type and timing of specimen collection and to determine the sensitivity and specificity of these tests.

As observed in this study, the creation of an integrated system of diagnostic laboratories — at the institutional, regional and national levels — was essential for an effective and rapid response to SARS, a new and emerging infectious disease. In addition, constant communication and cooperation between physicians on the front lines, epidemiologists and laboratory scientists has been crucial in managing and characterizing the Toronto SARS outbreak. These links must also be optimized at the level of laboratory, public health and hospital information systems to allow electronic data transfer between these groups. The lessons learned from the SARS outbreak highlight the importance of continued collaborations in the scientific and medical communities to prevent future outbreaks and minimize the impact of other new emerging infectious diseases.

This article has been peer reviewed.

From the University of Toronto (Tang, Louie, Richardson, Simor, Jamieson, Fearon, Poutanen, Mazzulli, Tellier, McGeer, Low, Phillips, Rachlis, Matukas, Walmsley, Gold); Sunnybrook and Women's College Health Sciences Centre (Tang, Louie, Simor, Phillips, Rachlis, Matukas); The Hospital for Sick Children, Toronto, Ont. (Richardson,Tellier); McMaster University, Hamilton, Ont. (Smieja, Mahony, Loeb, Petrich, Chernesky); Saint Joseph's Hospital, Hamilton, Ont. (Smieja, Mahony, Petrich, Chernesky); Central Public Health Laboratory, Toronto, Ont. (Jamieson, Fearon); Mount Sinai Hospital and Toronto Medical Laboratories, Toronto, Ont. (Poutanen, Mazzulli, McGeer, Low); National Microbiology Laboratory, Winnipeg, Man. (Jones, Bastien, Li, Dick, Grolla, Fernando, Booth); Toronto Public Health (Henry); The Scarborough Hospital, Scarborough, Ont. (Rose, Lovinsky); University Health Network, Toronto, Ont. (Walmsley, Gold); and St. Joseph's Health Centre, Toronto, Ont. (Krajden)

Competing interests: None declared.

Contributors: All authors contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, and the drafting and critical revision of the article. All authors approved the final version to be published.

Acknowledgements: We thank all the health care workers who cared for the patients described in this study. We also thank the staff at the Central Public Health Laboratory, the National Microbiology Laboratory and the participating hospital laboratories for their hard work during this outbreak.

This study was funded in part by the Ontario Ministry of Health and Long-Term Care and the Canadian Institute of Health Research. The results of this study were presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, held in Chicago in September 2003.

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e-Appendix 1: Primers used for reverse-transcriptase polymerase chain reaction (RT-PCR) for the detection of severe acute respiratory syndrome coronavirus (SARS-CoV)

Laboratory	Primers pairs	Gene target	Reference
National Microbiology Laboratory	RT-PCR primers 5′-CAGAGCCATGCCTAACATG-3′ 5′-AATGTTTACGCAGGTAAGCG-3′ Nested PCR primers 5′-TGTTAAACCAGGTGGAAC-3′ 5′-CCTGTGTTGTAGATTGCG-3′	Polymerase	Health Canada (Poutanen et al[5])
Central Public Health Laboratory	As above	As above	As above
Sunnybrook and Women's College Health Sciences Centre, Toronto	RT-PCR primers 5′-ATGAATTACCAAGTCAATGGTTAC-3′ 5′-CATAACCAGTCGGTACAGCTAC-3′ Nested PCR primers 5′-GAAGCTATTCGTCACGTTCG-3′ 5′-CTGTAGAAAATCCTAGCTGGAG-3′	Polymerase	RealArt™ HPA-Coronavirus LC RT PCR Kit (artus GmbH, Hamburg, Germany) (Drosten et al[3])
Hospital for Sick Children (HSC), Toronto	RT primer 5′- GCATAGGCAGTAGTTGCATC-3′ PCR primers 5′-TGATGGGATGGGACTATCCTAAGTGTGA-3′ 5′-TTGCATCACCACTAGTTGTGCCACCAGGTT-3′	Polymerase	HSC (Poutanen et al[5])
HSC, Toronto	5'-TGATGGGTTGGGACTATCCTAAATGTGA-3′ 5′-GTAGTTGCATCACCGGAAGTTGTGCCACC-3′	Polymerase	HSC (R. Tellier et al: unpublished data)
Saint Joseph's Hospital (SJH), Hamilton	5′-TGAATACACCCAAAGACCAC-3′ 5′-TGATGAGGAGCGAGAAGAG-3′	Nucleocapsid	SJH (J. Mahony et al: unpublished data)