Crouching Tiger, Hidden Dragon: The Laboratory Diagnosis of Severe Acute Respiratory Syndrome

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(See the brief report by Jiang et al. on pages 293–6)

While the world awaited the next influenza pandemic to emerge from southern China, "nature," as always, caught us by surprise. The unusual "atypical" pneumonia, subsequently called "severe acute respiratory syndrome" (SARS), that emerged out of southern China in late 2002 was not caused by influenza, but was, in fact, caused by a novel coronavirus [1–3]. Epidemiologically, infection with the SARS coronavirus (SARS-CoV) is closely linked with SARS [1, 4], and experimental infection of cynomolgus macaques *(Macaca fascicularis)* results in pathology reminiscent of the human disease [4, 5]. Human coronaviruses 229E and OC43 are known to be causes of the common cold and have received scant attention, either diagnostically or research wise, in the past few decades. Coronaviruses also cause disease in animals that varies from transmissible gastroenteritis in pigs, to feline infectious peritonitis in cats, to avian infectious bronchitis in chickens. However, phylogenetic relationships suggested that this new virus was not closely related to any of the previously known human or animal coronaviruses and that SARS-CoV may, in fact, be the first representative of a new group within the coronavirus family [6, 7]. The lack of serological evidence of prior SARS-CoV infection in the healthy population in many parts of the world, including regions where the disease outbreaks were the most severe, suggested that this was a virus that had recently entered the human population, presumably from an animal reservoir [1, 8]. Although the animal reservoir in nature remains to be defined, the recent isolation of SARS-CoV–like virus from small mammals in live wild-game animal markets in southern China confirmed the zoonotic origin of the virus and suggested that these markets could potentially be the interface where the inter-species jump from animals to humans occurs [9].

SARS is an acute pneumonic illness and is clinically difficult to distinguish from other types of atypical pneumonia in the absence of a clear epidemiological link to other patients with the disease [1, 10, 11]. Because of the propensity for transmission within hospitals, early diagnosis, isolation, and management was critical, and laboratory confirmation of the diagnosis early in the course of illness was vital. SARS-CoV infection is closely associated with disease and has rarely been detected in the absence of clinical disease. The question is how best to detect infection early in the course of the illness. Therein lies the dilemma.

As with other microbial infections, the options for diagnosis are the demonstration of the pathogen in clinical specimens or the demonstration of a serological response to the agent. Detection of seroconversion to SARS-CoV using immunofluorescence and well-validated ELISA tests has been a very reliable means for confirming the diagnosis. However, the antibody response appears only around day 10 of the illness, and, in some patients, it may take even longer [12]. Thus, serological testing still remains the means for retrospective diagnosis. To date, IgM or other subtype assays have not been useful for closing the diagnostic window within the first week of illness, although future research and development may improve matters. Virus culture is still insensitive, and, in any event, primary virus isolation takes too long to be clinically relevant. Options such as shell vial culture may be considerations in the future, when monoclonal antibody reagents become available. However, given the current recommendation that culture of SARS-CoV be restricted to biosafety level 3 containment, culture-based diagnostic techniques are unlikely to be widely available. Conventional RT-PCR and recently established

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real-time PCR technologies have been reported to detect this deadly virus in patients with SARS [1–3, 13–17].

These RT-PCR tests showed us that the virus is detectable in specimens obtained from the upper and lower respiratory tracts, including nasopharyngeal aspirates and throat swab specimens. Specimens obtained from the lower respiratory tract, including sputum samples [2], endotracheal aspirates [8], and bronchial lavage fluid, are excellent specimens for detection of SARS-CoV. However, few patients expectorate sputum during the early stage of the illness. Somewhat surprisingly, viral RNA was also detected in feces and urine samples, although urine was not the most sensitive specimen at any stage of the illness [8]. The suitability of serum or peripheral blood leukocytes as a diagnostic specimen remains to be examined. With the first-generation RT-PCR tests, the overall diagnostic yield in the second week of illness was >80%, and feces specimens yielded better results than did respiratory specimens [8]. By use of quantitative assays, it was shown that the virus load in the upper respiratory tract is low during the first week of illness and peaks around day 10 of illness [12]. Thus, in the first 5 days of illness, none of these specimens gave a satisfactory diagnostic yield, and it was clear that the low virus load at this stage of illness posed a diagnostic challenge. This may be partly because the virus targets the lower rather than the upper respiratory tract [10, 11]. In any event, when tests for diagnosis of SARS are compared, it is important to characterize specimens in relation to the duration of illness.

A number of approaches have been taken to address the challenge. In this issue of *Clinical Infectious Diseases,* Jiang et al. [18] describe a real-time nested PCR method for detection of SARS-CoV. With their assay, cDNA obtained from a throat swab sample was subjected sequentially to 2 rounds of amplification in a real-time quantitative PCR platform. The test is able to detect <10 copies of viral genome per reaction. Although only a small number of samples were evaluated, the assay seems to be more sensitive than a commercial, single-round RT-PCR kit. Discrepant results were all validated by serological testing, which demonstrated the specificity of their assay. The assay has a dynamic range from at least 10^3 to 1 copies per reaction and no detectable background signal from negative control samples. More importantly, the turn-around time for this method was much shorter than that for the conventional nested RT-PCR assay. However, the actual sensitivity and specificity of this method remains to be evaluated with a study involving a larger number of clinical specimens.

To develop a better diagnostic test for SARS, we take an alternative approach. For these reported assays, the target sequences are within the ORF1ab region. Because of the presence of a large amount of subgenomic viral mRNA in infected cells, one would expect that the detection rate of SARS-CoV might be increased by targeting subgenomic viral mRNA [6, 7]. To explore this possibility, with some colleagues, we recently compared the amounts of genomic and subgenomic RNA in clinical specimens [15]. To our surprise, our results indicated that genomic viral RNA is the predominant viral RNA species in clinical samples, suggesting that there is no advantage in targeting subgenomic mRNA sequences for clinical diagnosis [15].

In another recent study [14], with some colleagues, we demonstrated that improving specimen extraction alone can markedly improve the sensitivity of the RT-PCR assays and that, when the improved RNA extraction protocol was combined with an optimized and sensitive real time RT-PCR assay, a sensitivity of 80% can be achieved during the first 3 days of illness. A nasopharyngeal aspirate specimen (rather than a throat swab specimen) must be used in this assay for optimal results. Obtainment of nasopharyngeal aspirates or nasopharyngeal swab specimens from patients with SARS has sometimes been a cause for concern. However, with appropriate precautions, the procedures can be done safely, and these specimens are the best for the rapid diagnosis of infection with alternative pathogens, such as influenza virus, which are more likely to be the etiological agent in many suspected outbreaks of infection that are investigated during this winter season. Recently, Tsang et al. [16] demonstrated that positive RT-PCR results for nasopharyngeal aspirate samples were independent predictors of mortality, implying that virus loads might also be very useful for prognosis. Thus, quantitative RT-PCR assays may provide useful information for clinical management.

It is not possible to predict whether SARS will return. Given the likely existence of an animal reservoir, the reemergence of this disease remains possible. Its reemergence anywhere in the world, especially if it occurs during the winter with the cocirculation of influenza, will put intense pressure on health care services and diagnostic laboratories in many parts of the world. Although one hopes that such an eventuality will not arise, it is sensible to be prepared to meet such a challenge.

References

1. Peiris JS, Lai ST, Poon LLM, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet **2003**; 361:1319–25.
2. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med **2003**; 348:1967–76.
3. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. New Engl J Med 2003; 348:1953–66.
4. Kuiken T, Fouchier RA, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet **2003**; 362:263–70.
5. Fouchier RA, Kuiken T, Schutten M, et al. Aetiology: Koch's postulates fulfilled for SARS virus. Nature **2003**; 423:240.
6. Rota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science **2003**; 300:1394–9.
7. Marra MA, Jones SJ, Astell CR, et al. The genome sequence of the SARS-associated coronavirus. Science **2003**; 300:1399–404.
8. Chan KH, Poon LLM, Cheng VCC, et al. De-

tection of SARS coronavirus (SCoV) by RT-PCR, culture and serology in patients with severe acute respiratory syndrome (SARS). Emerg Infect Dis (in press).
9. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science **2003**; 302:276–8. Available at: http://www.sciencemag.org/cgi/content/full/302/5643/276.
10. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med **2003**; 348:1986–94.
11. Tsang KW, Ho PL, Ooi GC, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med **2003**; 348:1977–85.
12. Peiris JSM, Chu CM, Cheng VCC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet **2003**; 361:1767–72.
13. Poon LLM, Wong OK, Luk W, Yuen KY, Peiris JS, Guan Y. Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). Clin Chem **2003**; 49:953–5.
14. Poon LLM, Chan KH, Wong OK, et al. Early diagnosis of SARS coronavirus infection. J Clin Virol **2003**; 28:233–8.
15. Poon LLM, Chan KH, Wong OK, et al. Detection of SARS coronavirus in patients with SARS by conventional and real-time quantitative RT-PCR assays. Clin Chem (in press).
16. Tsang OT, Chau T, Choi K, et al. Coronavirus-positive nasopharyngeal aspirate as predictor for severe acute respiratory syndrome mortality. Emerg Infect Dis **2003**; 9:1381–7. Available at: http://www.cdc.gov/ncidod/eid/vol9no11/03-0400.htm.
17. Yam WC, Chan KH, Poon LLM, et al. Evaluation of reverse transcription–PCR assays for rapid diagnosis of severe acute respiratory syndrome associated with a novel coronavirus. J Clin Microbiol **2003**; 41:4521–4.
18. Jiang SS, Chen T, Yang J, et al. Sensitive and quantitative detection of severe acute respiratory syndrome coronavirus infection by real-time nested polymerase chain reaction. Clin Infect Dis **2003**; 38:293–6 (in this issue).