Detection of SARS Coronavirus in Plasma by Real-Time RT-PCR

TO THE EDITOR: The identification and sequencing of a novel coronavirus[1] associated with the recently described severe acute respiratory syndrome (SARS)[2] have permitted the development of antibody-based and genome-based tests for the infection.[3,4] Although antibody seroconversion provides reliable proof of infection, it is not suitable for early diagnosis. Techniques for genome detection have the potential to provide earlier diagnosis, but the sensitivity of genome-based tests of respiratory samples such as nasal and throat swabs is likely to be low in patients presenting early with fever alone, before the development of respiratory symptoms. We therefore investigated the potential of genome detection in blood to provide a diagnosis during this very early phase.

A total of 65 plasma samples obtained within 10 days after the onset of fever were available from 26 patients with SARS at the Prince of Wales Hospital, Hong Kong. All patients met the World Health Organization's case definition and had seroconversion or a fourfold increase in antibody to the SARS-associated coronavirus (SARS-CoV), as measured by immunofluorescence. Viral RNA was extracted and amplified by means of a nested reverse-transcriptase polymerase chain reaction (RT-PCR), with the use of both qualitative and quantitative real-time assays (ABI Prism 7000 system) and the primer sets described by Drosten et al.[4] Full details of the assay protocols are available as Supplementary Appendix 1 with the full text of this letter at www.nejm.org.

The rate of detection of SARS-CoV RNA in the 24 patients tested within three days after the onset of fever was 79 percent (detection in 19 of the 24 patients). Between 3 and 10 days after the onset of fever, no additional patients with viremia were identified with the use of either the qualitative or the quantitative assay (Fig. 1A). In the 19 patients in whom the viral genome could be quantified on one or more occasions, the plasma viremia level rose early and was maximal at around day 4 or 5 after the onset of fever (Fig. 1B). The viral load then decreased in most patients, though it remained detectable at a low level in four of the five patients from whom samples were obtained on either day 9 or day 10.

The detection sensitivity of 79 percent within the first three days is better than the reported rate for nasal and throat swabs and is equivalent to that for nasopharyngeal aspirates.[3] With the current protocol, RNA from only 70 μl of plasma was analyzed, and it is likely that centrifugation of a larger volume of plasma would increase the detection sensitivity. The use of plasma viremia for diagnosing SARS-CoV infection also has the advantage of not requiring nasopharyngeal aspiration, which is regarded as a risk-prone procedure.[5]

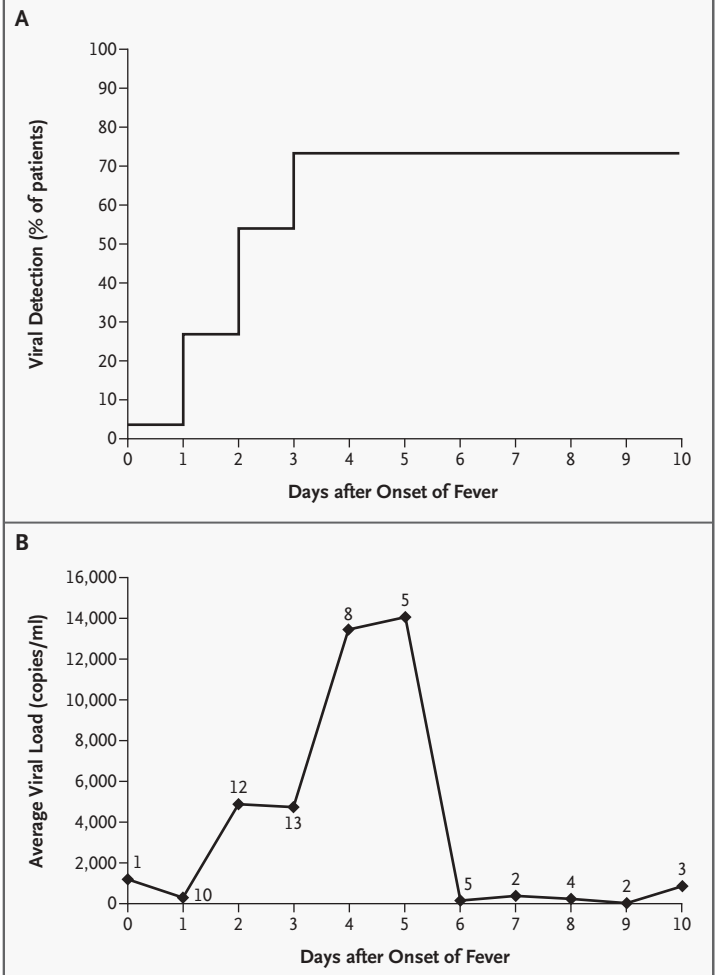

Figure 1. Detection of Severe Acute Respiratory Syndrome (SARS)–Associated Coronavirus with a Reverse-Transcriptase Polymerase-Chain-Reaction (PCR) Assay (Panel A) and Changes in the Viral Load (Panel B) in Plasma Samples Obtained up to 10 Days after the Onset of Fever in 26 Patients.

Viral infection was considered to have been detected if viral RNA was found by either the nested conventional or real-time PCR method. The number at each time point in Panel B is the number of samples tested.

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Estimated Timing of the Last Common Ancestor of the SARS Coronavirus

TO THE EDITOR: Using the viral sequences derived from clinical specimens collected in Hong Kong between February and April 2003 from 139 patients with the severe acute respiratory syndrome (SARS),[1] we attempted to estimate the timing of the last common ancestor of the coronavirus associated with the recent outbreaks of SARS. We previously reported the results of phylogenetic analysis, using the same set of spike-gene sequences as that in the SARS-associated coronavirus (SARS-CoV) isolated during the early phase of the outbreak (February to April) in Hong Kong and southern China, together with those available in the public data base.[1]

Assuming that the mutation rate was uniform, we carried out a linear regression analysis that was similar to the approach used for the timing of the origin of human immunodeficiency virus type 1.[2] The divergence (i.e., branch length), based on the genetic distance between the isolates and the root of the phylogram, was plotted as a function of the sampling time (Fig. 1).[2,4] The root was defined as the first diverging point[2] of the hypothetical common ancestor of SARS-CoV. The best-fit line was generated by the linear regression analysis extrapolated back to the past. The divergence of the sequences from the common ancestor was found to have a linear relation with time ($P<0.001$), in a progressive manner. The last appearance of the common ancestor of SARS-CoV was estimated to be on December 12, 2002 (95 percent confidence interval, September 26, 2002, to January 13, 2003), when the value of the divergence was zero. This finding suggests that the first deviation of the ancestral virus may have occurred in late 2002, close to the time of the first reports of SARS in southern China.[5] Although additional sampling of sequences, involving a longer time frame, is needed, the current findings shed some light on the origin of SARS-CoV and thus may help elucidate its evolution.

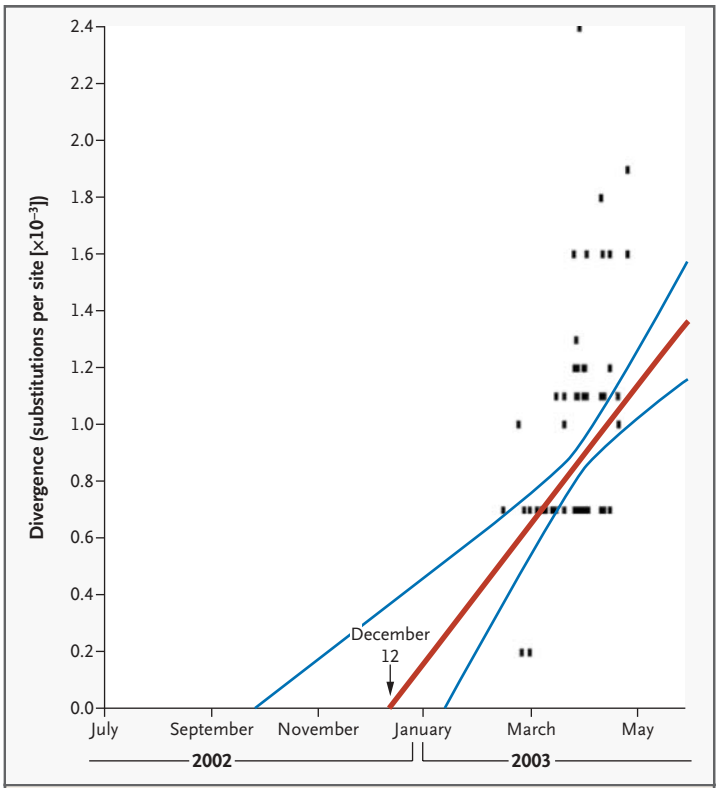

Figure 1. Timing of the Most Recent Common Ancestor of the Hong Kong SARS-CoV.

The divergence of the sequence from the ancestral sequence was plotted against sampling time (GraphPad Prism, version 4.00).[3] The red line represents the best-fit line obtained by linear regression analysis, and the blue lines indicate the 95 percent confidence intervals.