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Editorial

Interpreting Diagnostic Studies in SARS—Defining the Reference

Shortly after WHO issued the global alert about an outbreak of a severe atypical pneumonia without known etiology [later to be known as severe acute respiratory syndrome (SARS)], a case definition was developed [1,2]. This case definition, based on clinical criteria and epidemiologic risks, has since been the cornerstone by which SARS has been diagnosed.

Case definitions used for outbreak control are generally designed to be sensitive at the sacrifice of specificity, and primarily encompass the manifestations of the disease that are most responsible for transmission. The case definition for SARS, however, likely does not represent the entire spectrum of disease caused by SARS cornoavirus (SARS-CoV). Previous studies have documented that asymptomatic infection with SARS-CoV can occur; for example, asymptomatic health care workers who cared for patients with SARS developed antibodies to SARS-CoV [3,4]. Minimally symptomatic patients with epidemiologic risks, fevers, and antibodies to SARS-CoV, but no pulmonary symptoms or infiltrates on chest x-ray have also been reported [5].

In contrast, many acute respiratory illnesses in people meeting the case definitions for SARS (therefore necessarily having epidemiologic risks for SARS) have been proven to be caused by other viral pathogens such as influenza A or human metapneumovirus [6]. Although the case definition was critical in controlling the outbreak, its utility as the reference standard for evaluating tests is less clear.

In this issue, Wang et al. describe the development of a western blot with recombinant fragments of the SARS-CoV spike protein, and they attempt to show the utility of this test for the diagnosis of SARS. From a cohort of 20 patients who met the clinical case definition of SARS, three had a negative SARS ELISA. Two of the three were then shown by western blot to have antibodies to the S2 fragment. Although these results are intriguing, the difficulty is how to accurately interpret these findings.

There is much historical precedent for diagnostic serologies evolving from ELISA to western blot to improve sensitivity and/or specificity. The diagnosis of Lyme disease was first made by the presence of arthritis following the characteristic erythema migrans. With the recognition that erythema migrans may not be universally present, the ELISA was developed to confirm the diagnosis. However, the ELISA occasionally had false positives due to the presence of underlying diseases such as syphilis or relapsing fevers [7]. The western blot was developed to increase the specificity of using serology for the diagnosis of Lyme disease [8,9]. These studies validated the western blot by comparing it to a population with a typical history and the presence of erythema migrans (once considered the pathognomonic clinical marker for Lyme disease). It has subsequently been shown in small case series that cellulites, urticaria, and other dermatologic eruptions can be misdiagnosed as erythema migrans [10]. In addition, erythema migrans can be caused by infection with other *Borrelia* species that do not cause Lyme disease [11]. Although the western blot was validated against a population with symptoms of Lyme disease and erythema migrans, its sensitivity and specificity for diagnosing infection with *Borrelia burgdorferi* or Lyme disease without erythema multiforme are more difficult to define. Yet the western blot is considered to be the standard confirmatory serological test for Lyme (though the diagnosis can still be made by clinical presentation alone).

The difficulty in interpreting diagnostic studies for SARS is determining what control group should be selected as the reference. Comparison to a syndromic diagnosis risks involving a population that may not all have been infected with SARS-CoV. For this reason, it is difficult to interpret the meaning of the presence of antibodies to fragments of the spike protein in patients who meet the case definition yet have a negative ELISA. Furthermore, given the spectrum of diseases that SARS-CoV causes, validation of diagnostic studies against the case definition will be biased toward the more severe manifestations of the disease.

Newer immunologic techniques may help with this problem. The follow-up article in this issue by Wang et al. demonstrates the detection of cytotoxic T-lymphocyte (CTL) response against SARS-CoV. Even though this technique has been increasingly used in pathogenesis and vaccine response studies, there is little experience with its application as a diagnostic test. The CTL response defines a

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specific $CD8^+$ T cell effector function. It is a very complicated assay system with undefined sensitivity and specificity, so it is unclear if this test could become a reference standard. Alternatively, these newer tests may find utility in the prognostication of the infection, though at this juncture this is only supposition.

These papers demonstrate novel diagnostic test for SARS and provoke speculation about how the diagnosis of this infection will likely evolve. However, the true utility of these and other immunologic tests that could potentially be used to diagnose SARS will need to be validated with better prospective studies in which design obfuscates the necessary errors associated with the outbreak case definitions.

References

[1] WHO issues a global alert about cases of atypical pneumonia. Geneva: World Health Organization, 2003 (Accessed May 25, 2004, at http://www.who.int/mediacentre/releases/2003/pr22/en/).
[2] Case Definitions for Surveillance of Severe Acute Respiratory Syndrome (SARS) (Accessed May 25, 2004 at http://www.who.int/csr/sars/casedefinition/en/).
[3] P.C.Y. Woo, et al., Relative rates of non-pneumonic SARS coronavirus infection and SARS coronavirus pneumonia, Lancet 363 (2004) 841–845.
[4] H.K. Lee, E.Y. Tso, T.N. Chau, O.T. Tsang, K.W. Choi, T.S. Lai, Asymptomatic severe acute respiratory syndrome-associated coronavirus infection, Emerg. Infect. Dis. 9 (2003) 1491–1492.
[5] G. Li, Z. Zhao, L. Chen, Y. Zhou, Mild severe acute respiratory syndrome, Emerg. Infect. Dis. 9 (2003) 1182–1183.
[6] J.K. Louie, et al., SARS and Common Viral Infections, Emerg. Infect. Dis. 10 (2004) 1143–1146.
[7] L.A. Magnarelli, J.F. Anderson, R.C. Johnson, Cross-reactivity in serological tests for Lyme disease and other spirochetal infections, J. Infect. Dis. 156 (1) (1987, Jul) 183–188.
[8] S.M. Engstrom, E. Shoop, R.C. Johnson, Immunoblot interpretation criteria for serodiagnosis of early Lyme disease, J. Clin. Microbiol. 33 (2) (1995, Feb) 419–427.
[9] F. Dressler, J.A. Whelan, B.N. Reinhart, A.C. Steere, Western blotting in the serodiagnosis of Lyme disease, J. Infect. Dis. 167 (1993) 392–400.
[10] H.M. Feder Jr, D.L. Whitaker, Misdiagnosis of erythema migrans, Am. J. Med. 99 (1995) 412–419.
[11] S.G. Rijpkema, et al., Detection of Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia garinii and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans, Clin. Microbiol. Infect. 3 (1997) 109–116.

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