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Kinetics of Severe Acute Respiratory Syndrome (SARS) Coronavirus-Specific Antibodies in 271 Laboratory-Confirmed Cases of SARS

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The sensitivities and specificities of an immunofluorescence assay and an enzyme immunoassay for detection of antibodies specific for severe acute respiratory syndrome coronavirus (SARS-CoV) were compared for 148 laboratory-confirmed SARS cases. The appearance and persistence of SARS-CoV-specific antibodies were assessed, with immunoglobulin G detected in 59% of samples collected within 14 days and persisting for 60 to 95 days after the onset of illness.

Severe acute respiratory syndrome (SARS) is a new disease, with 8,422 probable cases reported by the World Health Organization as of 7 August 2003. Most cases have occurred in China (5,327 cases; the largest outbreak was in Beijing, with 2,521 cases), Taiwan, Hong Kong, Singapore, and Canada (http://www.who.int/csr/sars/country/2003_08_15/en/). Serological evidence of SARS coronavirus (SARS-CoV) infection has been found in most patients with symptoms fitting the clinical definition of SARS (2–4, 7, 9, 10), although data on the performance of various antibody detection assays and the persistence of SARS-CoV-specific antibodies are lacking.

The first aim of this study was to compare the sensitivities and specificities of a commercial immunofluorescence assay (IFA; Euroimmun AG, Lübeck, Germany) and a recombinant double-nucleocapsid antigen sandwich enzyme-linked immunosorbent assay (ELISA; Wantai Biological Pharmacy Enterprise Company, Ltd., Beijing, China) for SARS-CoV-specific immunoglobulin G (IgG) and IgM in patients for whom SARS-CoV RNA had been detected by reverse transcriptase PCR (RT-PCR). The second aim was to assess the timing of the appearance and persistence of SARS-CoV-specific antibodies after the onset of disease.

Three hundred four patients fitting the SARS clinical case definition (fever of 38°C or higher, cough or shortness of breath, new pulmonary infiltrates on chest radiography, and close contact with a person with a suspected or probable case) were hospitalized at Ditan Hospital, Beijing, China, between 26 March and 31 May 2003. Probable cases were regarded as laboratory confirmed if SARS-CoV-specific IgG and/or IgM was detected by IFA within 3 weeks of the onset of illness and/or SARS-CoV RNA was detected by RT-PCR within 2 weeks. SARS-CoV infection was laboratory confirmed in 271 of 304 (89.1%) cases, with 33 individuals testing SARS-CoV negative (10.9%; 27 of these had alternative laboratory diagnoses). The mean age of the 271 individuals with laboratory-confirmed cases was 36 ± 16 years, and they included 92 (33.9%) health care workers and 32 patients with significant underlying illnesses. SARS acquisition in the hospital setting, in either health care workers, inpatients, or hospital visitors, occurred in 112 (41.3%) cases, and a further 62 individuals acquired SARS following exposure at home to family members or friends with hospital-acquired infections. The clinical features were similar to those reported elsewhere (data not shown) (1, 3, 5, 10, 11).

Comparison of IFA and ELISA for detection of SARS-CoV-specific IgG and IgM. Testing was performed for 148 patients for whom SARS-CoV was detected in respiratory or fecal samples by RT-PCR. SARS-CoV IgM was detected for 117 (79%) people and SARS-CoV IgG was detected for 145 (98%) people by IFA, while IgM was detected for 133 (90%) people and IgG was detected for 120 (82%) people by ELISA. Controls included 105 asymptomatic close contacts of individuals with SARS cases (medical workers) and 90 individuals with chronic hepatitis B (30 cases), hepatitis C (30 cases), or human immunodeficiency virus type 1 (30 cases). No controls were found to have SARS-CoV antibodies by IFA, but SARS-CoV IgM was detected for one person each in the hepatitis B and hepatitis C groups, and IgG was detected for two of the human immunodeficiency virus-infected individuals by ELISA. Therefore, the overall sensitivity and specificity of SARS-CoV IgG detection by IFA for RT-PCR-positive patients were both 98%, compared to 81 and 99%, respectively, by ELISA. The positive predictive values (PPV) of the IFA and the ELISA for

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TABLE 1. Appearance of SARS-CoV-specific IgM and IgG antibodies by IFA in laboratory-confirmed cases of SARS

Days after onset	No. of samples	No. (%) of samples positive for SARS-CoV:		
		IgM	IgG	IgM + IgG
1–4	55	13 (23.6)	12 (16)	6 (8)
5–9	93	15 (16.1)	47 (50.5)	10 (10.8)
10–14	89	58 (65.2)	81 (91)	54 (60.7)
15–19	74	58 (78.4)	70 (94.6)	54 (73)
20–24	54	45 (83.3)	52 (96.3)	42 (79.6)
25–29	60	51 (85)	60 (100)	51 (85)
30–34	41	36 (87.8)	41 (100)	36 (87.8)
35–39	23	22 (95.7)	23 (100)	22 (95.7)
40–44	28	28 (100)	28 (100)	28 (100)
45–49	4	2 (50)	4 (100)	2 (50)
Over 50	9	3 (33.3)	9 (100)	3 (33.3)
Total	530	331 (62.5)	416 (78.5)	308 (58.1)

IgG detection were 100 and 98%, and the negative predictive values (NPV) were 98 and 87%, respectively. The sensitivity and specificity of SARS-CoV-specific IgM detection by IFA were 79 and 100%, respectively, compared to 90 and 99%, respectively, for ELISA IgM detection. The PPV of the IFA and ELISA for IgM detection were 100 and 99%, respectively, and the NPV were 86 and 93%, respectively. One hundred eighteen of 148 patients had SARS-CoV-specific IgG detected by both IFA and ELISA, 27 were IFA positive but ELISA negative, 2 were IFA negative but ELISA positive, and 1 was negative with both assays.

Appearance and persistence of SARS-CoV-specific antibodies after disease onset. Serial serum samples (total number, 530; 1 to 5 samples per patient) from 271 SARS patients for whom the collection dates and time of disease onset were available were tested for SARS-CoV-specific IgM and IgG by IFA (Table 1). Of 237 samples collected during the first 14 days of illness, SARS-CoV IgG was detected in 140 (59.1%) and SARS-CoV IgM was detected in 86 (36%). The level of detection increased during the second 15 days, so that 182 of 188 (96.9%) samples were seropositive for SARS-CoV IgG and 154 of 188 (81.9%) were seropositive for IgM. All 165 serum samples collected 25 days or more after disease onset were SARS-CoV IgG seropositive. SARS-CoV-specific IgM levels dropped as early as 2 or 3 weeks after the onset of illness (data not shown), in agreement with published data (6).

To examine antibody persistence after hospital discharge, samples from 70 patients were collected 60 to 95 days from the onset of illness and tested for the presence of SARS-CoV-specific IgG, IgM, and IgA by IFA. In all 70 (100%) SARS-CoV IgG was detected, with 58 (83%) showing titers over 100; in 58 (83%) SARS-CoV IgM was detected; and in 54 (77%) IgA was detected.

Since the clinical case definition of SARS is similar to that of other severe atypical pneumonias, laboratory tests that accurately diagnose SARS-CoV infection are important. SARS-CoV RT-PCR has only a modest sensitivity of approximately 30% on a single respiratory sample collected early in the illness (8), although its sensitivity improves if serial samples are collected in the first 2 weeks, since maximal viral shedding occurs 7 to 10 days after onset (4, 8). The most reliable laboratory test for the confirmation of SARS is detection of SARS-CoV-specific antibodies (4, 8). In Hong Kong, a prospective study of 75 SARS cases demonstrated SARS-CoV IgG seroconversion in 70 (93%) cases by use of an in-house indirect IFA with acetone-fixed SARS-CoV-infected Vero cells, with antibodies detectable at a mean of 20 days (range, 11 to 28) after onset (9). This level of detection was similar to that in the same group's retrospective analysis of 50 cases, where all 32 patients with acute- and convalescent-phase sera demonstrated seroconversion or fourfold rises in SARS-CoV-specific antibody levels by IFA; 280 control sera were negative (8). SARS-CoV-specific antibodies have been detected by IFA in 94% (264 of 282) of SARS cases in combined series (4). Seroconversion has usually occurred by 21 days, with IFA antibodies detected earlier by IFA than by ELISA (3, 8).

A number of in-house ELISA formats have been developed for SARS-CoV antibody detection, although published data comparing the sensitivity and specificity of these ELISAs with those of IFA and other antibody detection systems (Western blotting or neutralization) are limited. An ELISA antigen using detergent extraction and gamma irradiation of SARS-CoV-infected Vero E6 cells was able to detect seroconversion or antibody rises in a limited study of SARS patients from different countries, with the antibody detected 1 to 2 weeks after onset. In general, ELISA titers were higher than IFA titers, and SARS-CoV antibodies were not detected in sera from blood donors or people infected with OC43 or 229E human coronavirus (3). It is important that in-house or commercial ELISAs be validated against standard clinical case definitions and RT-PCR (or results confirmed), since interpretation of serosurveys may be difficult (12).

We have shown that 89.1% of patients in a single institution who fit a SARS clinical case definition had laboratory confirmation of SARS-CoV infection. The sensitivities and specificities of the SARS-CoV IgG assays, either the commercial IFA or the recombinant nucleocapsid antigen ELISA, were high in the context of a SARS outbreak and inspire confidence in their use should SARS recur. Importantly, the sensitivities and specificities of the antibody assays in this study were generated with SARS-CoV RT-PCR-positive cases rather than with cases identified only by a clinical definition of SARS (where other etiological agents may be present). Of interest is the fact that that SARS-CoV-specific antibodies were not found in 105 asymptomatic health care workers who came into close contact with SARS patients.

SARS-CoV IgG antibodies detected by IFA appeared early in the illness, with antibodies sometimes found within the first week. After 10 days of illness, more than 90% of samples were positive, confirming the findings of other studies (3, 6, 9) and showing the diagnostic utility of serology in SARS investigation.

The persistence of SARS-CoV antibodies is unknown but is important for interpretation of results should SARS reexposure occur, for completion of seroepidemiological studies, and for assessment of the efficacy of potential vaccines. We found that SARS-CoV IgG was detected in all patients 2 to 3 months after the onset of disease, although IgM and IgA seropositivity was lost in some individuals. Further longitudinal studies are needed to define SARS-CoV antibody persistence.

Data on different antigen preparation methods (such as

those for whole virus, infected-cell lysates, or recombinant antigens) for ELISA formats, the relative diagnostic value and timing of the appearance and persistence of different antibody types, the presence of antibodies in other body fluids, the influence of steroids or other therapy, and the value of rapid SARS-CoV antibody detection systems remain incomplete.

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