Supplementary materials

The procedures for ELISA and CMIA detection.

The measurement processes of ELISA and CMIA were conducted with an automatic ELISA analyser HB-300E (Jiaxing CRED Medical Equipment Co. Ltd., China) and automatic CMIA analyser Caris 200 (Xiamen UMIC Medical Instrument Co. Ltd., China) according to the manufacturer’s instructions, respectively. In brief, 100 μL of plasma sample was used for ELISA-Ab, and 10 μL of sample was added to 100 μL of sample diluent buffer for ELISA-IgM. For ELISA-IgG, the sample was 20-fold diluted with phosphate buffer, pH7.0, and 10μL of dilution was added to 100 μL sample diluent buffers. Then, the microplates were incubated at 37 ℃ for 30 min and washed for 5 times with washing buffer, followed by addition of 100 μl of enzyme reagent. After incubation at 37°C for 30 min, the microplates were washed with washing buffer for 5 times again, and 100 μL TMB substrate was added and incubated at 37 ℃ for 15 min. Then, the reaction was terminated by addition of 50 μL 2 M H2SO4 and the optical density at 450 nm with 630 nm as reference (OD450/630) was detected. The cut-off values of ELISA-Ab and ELISA-IgG was determined by mean OD value of negative controls Nc̅ plus 0.16, and if Nc was less than 0.03, it was replaced by 0.03. For ELISA-IgM, the cut-off value was calculated as Nc multiply by 2.1, and if the Nc was less than 0.05, 0.05 was used as Nc. To calculate the relative antibody binding signal, the OD value of each sample was divided by the corresponding cut-off value of each assay.

For CMIA-Ab, 50 μL of samples were mixed with 50 μL of paramagnetic microparticles coated with RBD, while for IgM-CMIA, 20 of μL sample was mixed with 50 μL of paramagnetic microparticles coated with anti-μ chain antibody and 50 μL sample dilution buffer. After incubation at 37 ℃ for 15 min, the mixtures were washed 2 times, and 100 μL of acridinium ester conjugated RBD were added, followed by incubation at 37 ℃ for 10 min. Then, the microparticles were washed for 4 times, and each 100 μL of pre-trigger solution (Hydrogen peroxide) and trigger solution (NaOH) were added and the relative light units (RLUs) was measured immediately. The cut-off value of CMIA-Ab was determined by RLUs of negative control plus (RLUs of positive control multiplied by 0.3), and that of CMIA-IgM was determined by RLUs of negative control plus (RLUs of positive control multiplied by 0.2).
Figure E1. Cumulative seroconversion rates since the onset of illness in 26 critical and 54 non-critical patients with COVID-19. (A) The curves of the cumulative seroconversion rates for total antibody. (B) The curves of the cumulative seroconversion rates for IgM. (C) The curves of the cumulative seroconversion rates for IgG. The total antibody, IgM and IgG were detected by ELISA, and the curves were plotted according to Kaplan-Meier method. The serological status of patients was assigned to be negative before the time that the first sample was collected, even if the first sample tested positive. There was no significant difference in the cumulative seroconversion rate and median seroconversion time between critical and non-critical groups as analyzed by the Kaplan-Meier method.
Figure E2. Cumulative seroconversion rates since the exposure of SARS-CoV-2 in 15 critical and 30 non-critical patients with COVID-19. (A) The curves of the cumulative seroconversion rates for total antibody. (B) The curves of the cumulative seroconversion rates for IgM. (C) The curves of the cumulative seroconversion rates for IgG. The total antibody, IgM and IgG were detected by ELISA, and the curves were plotted according to Kaplan-Meier method. The serological status of the patient was assigned to be negative before the time that the first sample was collected, even if the first sample tested positive. There was no significant difference in the cumulative seroconversion rate and median seroconversion time between critical and non-critical groups as analyzed by the Kaplan-Meier method.