MATERIALS AND METHODS

Study design and population

We conducted a retrospective cohort study on 374 COVID-19 patients hospitalized in the isolation wards of Nanjing Public Health Medical Center (part of the Second Hospital of Nanjing) from July to August, 2021. All the patients had been exposed to COVID-19 in Jiangsu Province caused by SARS-CoV-2 B.1.617.2 (Delta) variant. China has adopted a dynamic zero-COVID policy. With this strategy, the surveillance system could efficiently track all the related cases after a local outbreak of COVID-19. In our study, samples from patients with COVID-19 were sequenced by the local Centers for Disease Control and Prevention, if the SARS-COV-2 PCR cycle threshold (Ct) value was less than 30. All subjects were confirmed to have an epidemiological link with the sequencing-confirmed cases infected with the Delta variant. Medical records, including demographic, clinical and laboratory data, were collected from the electronic health record system. Patients were included if they: (1) aged 18 years or more; (2) fulfilled the definition of vaccination in this study; (3) showed positivity in at least one SARS-CoV-2 nucleic acid test during hospitalization; (4) exhibited baseline SARS-COV-2 IgG levels that to some extent reflected the immunological responses to SARS-COV-2. This study was approved by the ethics committee of the Second Hospital of Nanjing (reference number: 2020-LS-ky003). Written informed consent was waived by the Ethics Commission.

SARS-COV-2 nucleic acid detection

Nasopharyngeal swab specimens were immersed in cell preservation solution (X1003, Sansure Biotech, Hunan, China) and immediately transported to the clinical diagnostic lab in Nanjing Public Health Medical Center. Then, the total nucleic acids were extracted from 200 μL of cell preservation solution using an automated nucleic acid extraction system (BioPerfectus Technologies Company, Jiangsu, China). SARS-COV-2 nucleic acid was then measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) kits (Sansure Biotech, Hunan, China). The cycle threshold (Ct) value from qRT-PCR was used to relatively represent the viral load. A Ct value more than 40 was considered negative. Generally, the viral load was monitored every day (no longer than 2 days) if the Ct value exceeded 30. To prevent over-estimation on the duration of viral shedding caused by any delayed SARS-CoV-2 nucleic acid test, the patients with suboptimal viral load monitoring were excluded from analysis.

SARS-COV-2 antibody tests

The levels of SARS-COV-2 IgM and IgG antibodies were measured using 2019-nCOV IgM and IgG antibody detection kits, respectively (BiOSCiENCE, Tianjin, China). The reagents were based on magnetic-particle-based chemiluminescent immunoassays targeting SARS-COV-2 spike receptor-binding domain (RBD). The serum samples were collected and analyzed by an automated chemiluminescent immunoassay system (Axceed 260, BiOSCiENCE, Tianjin, China). A signal/cut-off (S/CO) value ≥ 1 was considered positive.

Definition

The viral clearance was defined as two consecutive negative SARS-COV-2 nucleic acid tests with an interval of at least 24 hours. In this condition, the time of the first negative test was defined as the day of viral clearance. The duration of viral shedding was the interval between the time points of disease onset and viral clearance. Disease onset time point was defined when symptoms first appeared, or when SARS-CoV-2 nucleic acid was positive for the patients without symptoms. Viral clearance achieved within three weeks was considered “early viral clearance”. The three weeks, a cut-off value used for differentiation of early *vs* delayed viral clearance, was close to the first quantile of duration of viral shedding (20 days) in the study population.

Unvaccinated patients were those who had not received any COVID-19 vaccine before the diagnosis of COVID-19. Fully vaccinated patients were those who had received 2 doses of inactivated COVID-19 vaccines with disease setting on within at least 14 days after the second dose. A single dose of inactivated COVID-19 vaccine given at least 14 days before the disease onset was considered as partial vaccination. To avoid ambiguity in definition, the patients who received their first dose within 14 days or their second dose within 14 days before the disease onset were not included in our study. The patients that had received other strains of COVID-19 vaccines were also excluded.

The severity of COVID-19 was clarified based on the “Guideline of COVID-19 Diagnosis and Treatment (trial version 8)” issued by the National Health Council of China. Severe

COVID-19 should meet one of the following criteria: (1) respiratory rate ≥30 breaths/min, (2) at rest, the oxygen saturation of fingers while breathing air ≤93%, (3) arterial partial pressure of oxygen (PaO2)/oxygen uptake concentration (FiO2) ≤300 mmHg. If one patient developed respiratory failure requiring mechanical ventilation or had evidence of shock or other organ dysfunctions that needed to be treated at the intensive care unit (ICU), critical COVID-1 was considered 9[46]. Other conditions were grouped as non-severe COVID-19.

Statistical analysis

Continuous variables were expressed as medians and interquartile ranges (IQR). Categorical variables were summarized as counts and percentages in each category. Between-group comparison was done using the Mann–Whitney U test for continuous variables, and Pearson Chi-Square test or McNemar's test for categorical variables as appropriate. Multi-group comparison was performed using Kruskal-Wallis test, jonckheere-terpstra test or Pearson Chi-Square test. Spearman's rank correlation coefficient was used to measure correlation between two continuous variables. The time to achieve the first of two consecutive negative SARS-COV-2 nucleic acid tests was portrayed by Kaplan–Meier plot. Factors related to early viral clearance were analyzed by binary logistic regression analysis, and the relationship was expressed with odds ratio (OR) and 95% confidence interval (95% CI). A P value of less than 0.05 was considered statistically significant. All analyses were performed using R software for Windows version 4.0.5 (https://www.r-project.org/).