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Research article

Impact of antibody-level on viral shedding in B.1.617.2 (Delta) variant-infected patients analyzed using a joint model of longitudinal and time-to-event data

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Abstract: Knowledge of viral shedding remains limited. Repeated measurement data have been rarely used to explore the influencing factors. In this study, a joint model was developed to explore and validate the factors influencing the duration of viral shedding based on longitudinal data and survival data. We divided 361 patients infected with Delta variant hospitalized in Nanjing Second Hospital into two groups (⩽ 21 days group and > 21 days group) according to the duration of viral shedding, and compared their baseline characteristics. Correlation analysis was performed to identify the factors influencing the duration of viral shedding. Further, a joint model was established based on longitudinal

data and survival data, and the Markov chain Monte Carlo algorithm was used to explain the influencing factors. In correlation analysis, patients having received vaccination had a higher antibody level at admission than unvaccinated patients, and with the increase of antibody level, the duration of viral shedding shortened. The linear mixed-effects model showed the longitudinal variation of logSARS-COV-2 IgM sample/cutoff (S/CO) values, with a parameter estimate of 0.193 and a standard error of 0.017. Considering gender as an influencing factor, the parameter estimate of the Cox model and their standard error were 0.205 and 0.1093 (P = 0.608), the corresponding OR value was 1.228. The joint model output showed that SARS-COV-2 IgM (S/CO) level was strongly associated with the risk of a composite event at the 95% confidence level, and a doubling of SARS-COV-2 IgM (S/CO) level was associated with a 1.38-fold (95% CI: [1.16,1.72]) increase in the risk of viral non-shedding. A higher antibody level in vaccinated patients, as well as the presence of IgM antibodies in serum, can accelerate shedding of the mutant virus. This study provides some evidence support for vaccine prevention and control of COVID-19 variants.

Keywords: COVID-19; SARS-CoV-2; viral shedding; joint model; antibodies

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in December 2019 in Wuhan, China [1]. The COVID-19 pandemic has brought with over 528 million cases and more than 6.29 million confirmed deaths, overwhelming the healthcare system worldwide [2]. As the pandemic progresses, virus variants have showed up unabatedly. The B.1.617.2 (Delta) variant has a higher transmissibility and a stronger immune evasion capacity than B.1.1.7 (Alpha), B.1.351 (Beta) and P.1 (Gamma) variants [3], and its high infectivity is associated with a high viral load and a short incubation period [4]. The Delta variant shows many mutations in the spike protein, which can bind to the angiotensin converting enzyme 2 (ACE2) receptor, thus contributing to the fusion and integration of the virus with the host cell [5]. As one of the two currently circulating variants of concern (VOCs) [6], the public health threat posed by Delta variant around the world cannot be underestimated.

Viral clearance in a COVID-19 patient is defined as two consecutive negative (polymerase chain reaction) PCR results with an interval of at least 24 hours [7]. A longer viral shedding indicates a worse prognosis of COVID-19 patients [8]. Some studies observed a significant increase in antibodies against spike protein after vaccination and a positive correlation with the level of 50% neutralizing titer [9,10]. There was a remarkably strong non-linear relationship between the mean neutralization level and the protective effect of vaccines [11]. Vaccines are effective to reduce the odds of hospitalization and severe disease due to the Delta variant [4]. Some studies adapted a cohort design to investigate the relationship between disease severity and viral shedding, found that the more severe the disease, the longer the viral shedding time [12–14]. A retrospective study with 410 COVID-19 patients showed that coronary heart disease (CHD), albumin level, and time of initial antiviral treatment all impacted viral shedding time [15]. Another retrospective cohort study found that delayed admission to hospital after illness onset and male sex were associated with prolonged SARS-CoV-2 RNA shedding [16]. Also, an observational, retrospective, single-centre study found that viral clearance was negatively with respiratory disease severity, comorbidities and delayed hospital admission [17]. Besides, a

prospective study observed prolonged viral shedding in older, female and those with longer interval from symptom onset to admission [18].

Gong et al. [19] performed a simple correlation analysis of viral shedding and antibody level under a retrospective cohort of 564 participants, but found that viral shedding duration was not significantly correlated with antibody concentration. However, in this correlation analysis, the dynamic change of antibody level was not considered for exploring its influence on the duration of virus shedding. Significantly reduced duration of infectious viral shedding has been found among vaccinated individuals compared with unvaccinated individuals with a difference test [20]. But it failed to fully exploit longitudinal data on individual antibody levels to dynamically predict the timing of viral shedding in individuals. The joint model (JM) is a popular tool to process time-depending variables [21], which combines the mixed model or random effect model into the Cox model to construct the relationship between longitudinal covariates and the duration of an event [22]. Therefore, we monitored the dynamic change in antibody levels of hospitalized patients, and quantitatively analyzed its influence on the duration of viral shedding by using the JM model.

In this study we analyzed the effect of the vaccine on the patient's antibody levels at first admission using correlation analysis, then explored the relationships between prolonged viral shedding and the antibody level in patients infected with COVID-19 using JM based on repeated measurement indicators. Our findings could provide some theoretical support for the effectiveness and application of vaccines.

2. Materials and methods

2.1. Study participants

From July to August, 2021, we recruited a group of patients diagnosed with COVID-19 caused by SARS-CoV-2 B.1.617.2 (Delta) variant in Nanjing Second Hospital, the designated hospital for COVID-19 treatment in Jiangsu Province of China. The clinical classification was based on the "New Coronavirus Pneumonia Prevention and Control Program (Eighth Edition)". Enrollment criteria for this study were as follows: a. positive RT-PCR test for COVID-19 at admission; b. disappearance of symptoms after standardized treatment in the hospital; c. age $\geqslant$ 18 years old. This study was approved by the ethics committee of the Second Hospital of Nanjing (reference number: 2020-LS-ky003). Due to the anonymous processing of all patient private information in the article, the informed consent was waived with the approval of the Ethics Committee of Nanjing Medical University. Demographics, clinical and laboratory parameters, treatment management and outcome data were derived from the patients' medical history.

Initially, 544 patients were recruited, and 27 patients aged < 18 years old were excluded. According to the definition of viral shedding, 143 patients lacking nucleic acid test information within 48 hours were also excluded. In further analysis, 13 patients with outliers were excluded (Figure 1). Finally, 361 patients were included.

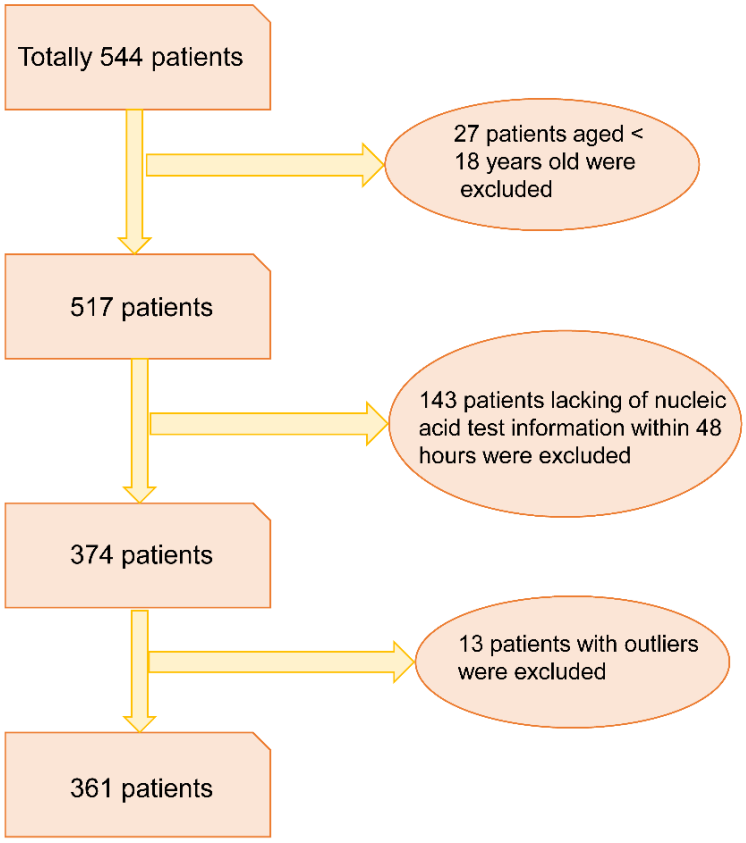

Figure 1. Flow diagram of data processing.

2.2. Statistical analysis

The joint model is constructed to infer the dependence and degree of correlation between longitudinal data and survival data, thus enabling a better assessment of the effectiveness of a decision or treatment measure [22,23]. The joint model has two basic components: the longitudinal submodel and the survival submodel.

In this study, we assumed a generalized linear mixed-effects model with the following structure:

$$g[E\{y_i(t) \mid b_i\}] = \eta_i(t) = x_i^T(t)\beta + z_i^T(t)b_i \tag{1}$$

where $g(\cdot)$ denoted a known one-to-one monotonic link function, $y_i(t)$ denoted the value of the ith subject at time point t, $\eta_i(t)$denoted the true level of potential longitudinal measurement values at time t and $x_i(t)$ and $z_i(t)$ denoted the design vectors for the fixed effects β and the random effects b_i. b_i was assumed to follow a multivariate normal distribution with mean zero and variance-covariance matrix **D**. For the survival submodel, we assumed that the risk of an event at moment t depended on an individual-specific linear predictor function $\eta_i(t)$. Specifically, we had

$$\begin{aligned} h_i(t \mid \mathcal{H}_i(t), \omega_i(t)) &= \frac{\lim_{\Delta t \to 0} \Pr\{t \le T_i^* < t+\Delta t \mid T_i^* \ge t, \mathcal{H}_i(t), \omega_i(t)\}}{\Delta t}, t > 0 \\ &= h_0(t)\exp\left[\gamma^\top \omega_i(t) + f\{\mathcal{H}_i(t), b_i, \alpha\}\right], \end{aligned} \tag{2}$$

where $\mathcal{H}_i(t) = \{\eta_i(s), 0 \le s \le t\}$ denoted the history of the underlying longitudinal process up to t for subject i. $h_0(\cdot)$ denoted the baseline hazard function and $\omega_i(t)$ was covariates (exogenous, possibly time-varying) with corresponding regression coefficients γ.

$$f\{\mathcal{H}_i(t), b_i, \alpha\} = \alpha\eta_i(t), \tag{3}$$

where $\mathcal{H}_i(t) = \{\eta_i(s), 0 \le s \le t\}$ denoted the true observed longitudinal process trajectory up to time point t for subject i, and $h_0(\cdot)$ denoted the baseline hazard function. $\omega_i(t)$ was a vector of covariates, and γ was the regression coefficient. The longitudinal and survival submodels were connected by the parameter α, which quantified the effect of potential longitudinal outcomes on event risk. The baseline hazard function $h_0(\cdot)$ was usually not specified in the standard Cox model, but $h_0(\cdot)$ needed to be explicitly defined in the joint model. The baseline hazard function $h_0(\cdot)$ was flexibly modeled using a B-splines approach,

$$\log h_0(t) = \gamma_{h_0,0} + \sum_{q=1}^{Q} \gamma_{h_0,q} B_q(t, v), \tag{4}$$

$B_q(t, v)$ denoted the qth basis function 1, $\cdots$, v_Q and γ_{h_0} spline coefficient vector of the B-splines with v as the node, usually Q = 15 or 20.

The Bayesian approach was used to develop a joint model for longitudinal and survival data, and the estimation method followed the Markov chain Monte Carlo (MCMC) algorithm. The JMbayes package in R was implemented. The theoretical development of the posterior distribution was based on the assumption that both longitudinal and survival processes were independent under the influence of a given random effect. In addition, the longitudinal response needed to consider the general assumption of independence of random effects. If θ denoted the set of all fixed parameters and b denoted the set of random parameters, , it was possible to determine the probability density function $p(\cdot)$ as

$$p(y_i, T_i, \delta_i \mid b_i, \theta) = p(y_i \mid b_i, \theta) p(T_i, \delta_i \mid b_i, \theta), \tag{5}$$

and

$$p(y_i \mid b_i, \theta) = \prod_l p(y_{il} \mid b_i, \theta), \tag{6}$$

Under these assumptions, the posterior distribution was similar to

$$p(\theta, b) \propto \prod_{i=0}^{n} \prod_{l=1}^{n_i} p(y_{il} \mid b_i, \theta) p(T_i, \delta_i \mid b_i, \theta) p(b_i \mid \theta) p(\theta). \tag{7}$$

3. Results

3.1. Baseline description

After data processing, 361 patients were included. The outliers and null values were imputed, the most frequent values were used to impute categorical variables, while the mean values were used to express continuous variables. Based on the fact that the duration of viral shedding was generally 2 or 3 weeks in different variants, and the median time of viral shedding in this study is 25 days, we chose 3 weeks as the time point of the viral shedding group. We divided the patients into two groups

according to the duration of viral shedding (⩽ 21 days group and > 21 days group). Patients' characteristics were compared between the two groups, categorical variables were expressed as frequency (%), and continuous variables were expressed as medians (with interquartile range [IQR]). The statistically significant level was set at 0.05 (Table 1). No obvious difference was found in the median age between the two groups (49 years vs. 50 years, p = 0.101). Female patients accounted for a higher proportion than males. Among all patients, 104 (28.8%) suffered basic diseases (such as hypertension, diabetes, heart attack, and tumor), and the rate of basic diseases showed no difference between the two groups (p = 0.706). Totally, 240 patients (66.5%) received vaccination, and the impact of vaccination on the duration of viral shedding was obvious (p = 0.046). The median time from illness onset to hospitalization was about 2 days. The main symptoms were fever, cough, sputum production and shortness of breath. The blood laboratory indicators included C-reactive protein (mg/L), procalcitonin (ng/mL), interleukin-6 (pg/mL), white blood cell count (109/L), neutrophil count (109/L), lymphocyte count (109/L), hemoglobin(g/L), platelet count(109/L), albumin(g/L), total bilirubin (umol/L), AST (U/L), ALT (U/L), urea nitrogen (mmol/L), creatinine (mol/L), eGFR (ml/min), creatine kinase (U/L), CK-MB (ng/mL), myoglobin (ng/mL), troponin I (pg/mL), LDH (U/L), prothrombin time (s), D-dimer (mg/L), and FDPs (ug/mL) (Table1). Two types of antibodies, SARS-COV-2 IgM sample/cutoff (S/CO) and SARS-COV-2 IgG (S/CO), were included in this study and the patient's antibody level at admission showed obvious difference between the two groups. Bar plot of the number of new admissions stratified by vaccination status was drawn (Figure 2). Since 20 July, the number of cases admitted to hospital has fluctuated. The peak was between August 2 and August 4, and the proportion of unvaccinated people on these three days was relatively high.

Table 1. Baseline characteristics of Delta COVID-19 patients.

	Overall (n =361)	Duration of viral shedding ≤ 21d (n =111)	Duration of viral shedding > 21d (n =250)	*P*-value
Demographics and clinical characteristics				
Sex				
Male	139 (38.5)	47 (42.3)	92 (36.8)	0.349
Female	222 (61.5)	64 (57.7)	158 (63.2)	
Age, years	50.00 [40.00, 65.00]	49.00 [34.50, 62.00]	50.00 [41.00, 66.00]	0.101
With any comorbidity				
No	257 (71.2)	81 (73.0)	176 (70.4)	0.706
Yes	104 (28.8)	30 (27.0)	74 (29.6)	
Hypertension				
No	284 (78.7)	90 (81.1)	194 (77.6)	0.489
Yes	77 (21.3)	21 (18.9)	56 (22.4)	
Diabetes				
No	330 (91.4)	105 (94.6)	225 (90.0)	0.221
Yes	31 (8.6)	6 (5.4)	25 (10.0)	
Heart disease				
No	349 (96.7)	106 (95.5)	243 (97.2)	0.525
Yes	12 (3.3)	5 (4.5)	7 (2.8)	

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	Overall (n =361)	Duration of viral shedding ≤ 21d (n = 111)	Duration of viral shedding > 21d (n = 250)	*P*-value
COPD				
No	361 (100.0)	111 (100.0)	250 (100.0)	
Yes	0(0.0)	0(0.0)	0(0.0)	
Carcinoma history				
No	353 (97.8)	111 (100.0)	242 (96.8)	0.113
Yes	8 (2.2)	0 (0.0)	8 (3.2)	
Asthma				
No	356 (98.6)	111 (100.0)	245 (98.0)	0.329
Yes	5 (1.4)	0 (0.0)	5 (2.0)	
Autoimmune diseases				
No	357 (98.9)	110 (99.1)	247 (98.8)	1.000
Yes	4 (1.1)	1 (0.9)	3 (1.2)	
Vaccination Status				
Unvaccinated	121 (33.5)	29 (26.1)	92 (36.8)	0.046
Partially vaccinated	64 (17.7)	17 (15.3)	47 (18.8)	
Fully vaccinated	176 (48.8)	65 (58.6)	111 (44.4)	
Time from illness onset to hospitalization (median [IQR])	2.00 [1.00, 4.00]	2.00 [1.00, 4.00]	2.00 [1.00, 4.00]	0.192
Symptoms				
Fever				
No	247 (68.4)	77 (69.4)	170 (68.0)	0.902
Yes	114 (31.6)	34 (30.6)	80 (32.0)	
Cough				
No	179 (49.6)	56 (50.5)	123 (49.2)	0.909
Yes	182 (50.4)	55 (49.5)	127 (50.8)	
Sputum production				
No	294 (81.4)	90 (81.1)	204 (81.6)	0.885
Yes	67 (18.6)	21 (18.9)	46 (18.4)	
Shortness of breath				
No	349 (96.7)	109 (98.2)	240 (96.0)	0.357
Yes	12 (3.3)	2 (1.8)	10 (4.0)	
Nausea or vomiting				
No	355 (98.3)	110 (99.1)	245 (98.0)	0.671
Yes	6 (1.7)	1 (0.9)	5 (2.0)	
Abdominal pain or diarrhea				
No	340 (94.2)	103 (92.8)	237 (94.8)	0.470
Yes	21 (5.8)	8 (7.2)	13 (5.2)	

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	Overall (n =361)	Duration of viral shedding ≤ 21d (n = 111)	Duration of viral shedding > 21d (n = 250)	P-value
Loss of smell or taste				
No	347 (96.1)	105 (94.6)	242 (96.8)	0.377
Yes	14 (3.9)	6 (5.4)	8 (3.2)	
Myalgia				
No	351 (97.2)	108 (97.3)	243 (97.2)	1.000
Yes	10 (2.8)	3 (2.7)	7 (2.8)	
Stuffy nose or runny nose				
No	311 (86.1)	92 (82.9)	219 (87.6)	0.249
Yes	50 (13.9)	19 (17.1)	31 (12.4)	
Headache or dizziness				
No	334 (92.5)	106 (95.5)	228 (91.2)	0.195
Yes	27 (7.5)	5 (4.5)	22 (8.8)	
Fatigue				
No	291 (80.6)	93 (83.8)	198 (79.2)	0.387
Yes	70 (19.4)	18 (16.2)	52 (20.8)	
Pharyngeal discomfort				
No	280 (77.6)	91 (82.0)	189 (75.6)	0.219
Yes	81 (22.4)	20 (18.0)	61 (24.4)	
Blood laboratory findings				
C-reactive protein, mg/L	5.41 [2.04, 14.31]	5.77 [1.31, 17.01]	5.21 [2.52, 13.53]	0.868
Procalcitonin, ng/mL	0.04 [0.02, 0.06]	0.04 [0.02, 0.07]	0.04 [0.03, 0.06]	0.583
Interleukin-6, pg/mL	10.06 [2.81, 21.13]	10.69 [4.54, 21.02]	9.80 [2.33, 20.95]	0.453
White blood cell count, × 10^9/L	4.82 [3.91, 5.98]	4.79 [3.84, 6.30]	4.85 [3.94, 5.90]	0.918
Neutrophil count, × 10^9/L	3.00 [2.19, 4.02]	2.94 [2.06, 4.19]	3.04 [2.28, 3.86]	0.679
Lymphocyte count, × 10^9/L	1.15 [0.88, 1.54]	1.23 [1.00, 1.60]	1.11 [0.86, 1.47]	0.017
Hemoglobin, g/dL	13.20 [12.30, 14.60]	13.40 [12.35, 14.85]	13.10 [12.30, 14.50]	0.352
Platelet count, × 10^9/L	157.00 [121.00, 192.00]	165.00 [130.00, 200.50]	152.00 [118.25, 189.00]	0.102
Albumin, g/L	42.80 [40.40, 45.30]	43.10 [40.60, 45.45]	42.70 [40.20, 45.20]	0.261
Total bilirubin, umol/L	9.70 [7.60, 12.30]	9.90 [7.75, 12.10]	9.50 [7.53, 12.57]	0.981
AST, U/L	17.00 [13.90, 23.10]	16.10 [13.65, 23.30]	17.30 [14.35, 23.10]	0.189
ALT, U/L	18.10 [12.20, 28.50]	19.50 [12.00, 30.80]	17.40 [12.35, 28.10]	0.702
Urea nitrogen, mmol/L	4.36 [3.59, 5.24]	4.33 [3.62, 5.22]	4.40 [3.59, 5.27]	0.615
Creatinine, μmol/L	60.80 [51.90, 74.90]	63.50 [54.80, 76.50]	59.15 [51.12, 74.12]	0.045
eGFR, mL/min	111.95 [105.75, 117.14]	111.95 [104.68, 119.67]	111.95 [106.93, 115.50]	0.944

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	Overall (n = 361)	Duration of viral shedding ≤ 21d (n = 111)	Duration of viral shedding > 21d (n = 250)	P-value
Creatine kinase, U/L	71.00 [50.00, 112.00]	71.00 [51.50, 104.00]	69.50 [50.00, 115.75]	0.698
CK-MB, ng/mL	0.50 [0.30, 0.80]	0.50 [0.30, 0.85]	0.50 [0.30, 0.80]	0.990
Myoglobin, ng/mL	34.05 [23.60, 49.50]	34.05 [25.80, 49.90]	34.05 [22.22, 48.98]	0.464
Troponin I, pg/mL	3.00 [1.00, 6.20]	3.00 [0.80, 5.45]	3.00 [1.10, 6.40]	0.580
LDH, U/L	237.00 [202.00, 280.00]	234.00 [200.00, 269.50]	238.50 [206.00, 284.00]	0.251
D-dimer, mg/L	0.39 [0.25, 0.60]	0.40 [0.25, 0.67]	0.38 [0.25, 0.60]	0.957
FDPs, ug/mL	2.70 [1.70, 5.10]	3.10 [1.55, 5.40]	2.70 [1.70, 4.10]	0.242
SARS-COV-2 IgM (S/CO)	0.19 [0.06, 0.75]	0.34 [0.11, 1.36]	0.15 [0.05, 0.60]	<0.001
SARS-COV-2 IgG (S/CO)	1.13 [0.15, 7.61]	3.31 [0.38, 25.44]	0.67 [0.11, 3.95]	<0.001

Abbreviations: AST, Aspartate Transaminase; ALT, Alanine Aminotransferase; eGFR, estimated Giomerular Filtration Rate; CK-MB, Creatine Kinase Isoenzyme; LDH, Lactic Dehydrogenase; FDPs, Fibrinogen Degrdtion Products; SARS-COV-2 IgM, Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin M; SARS-COV-2 IgG, Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin G.

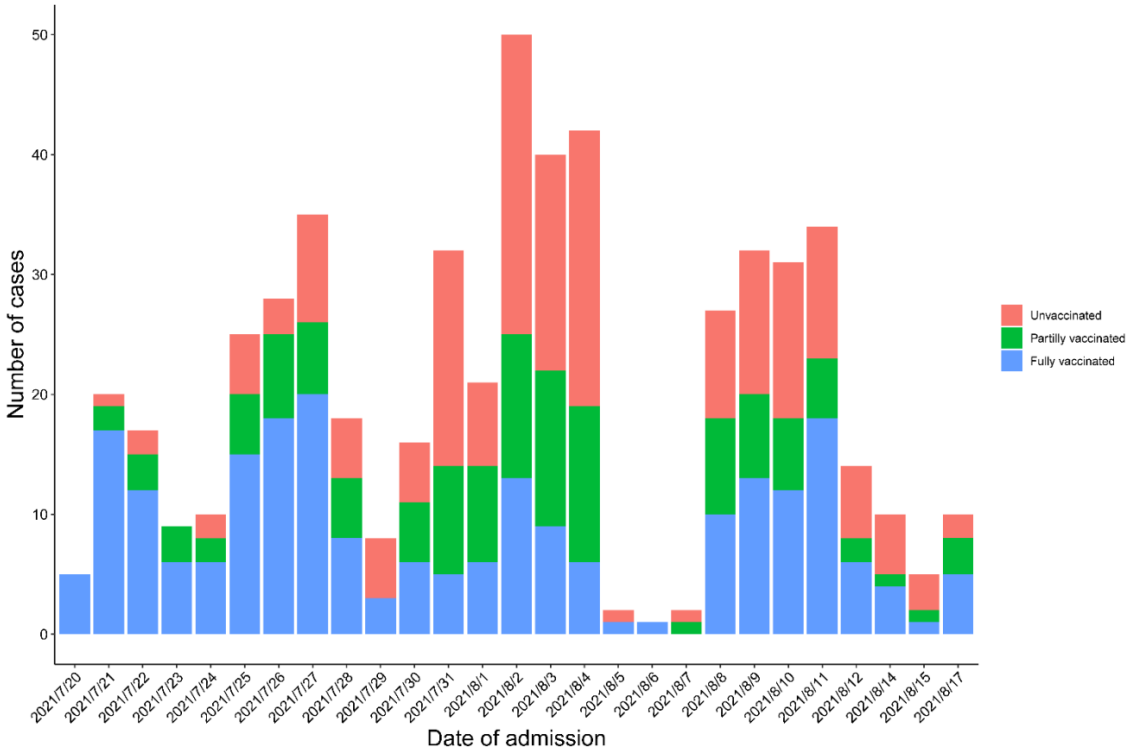

Figure 2. Bar plot of the number of new admissions stratified by vaccination status.

3.2. Correlation analysis

The median interval between a patient's last vaccination and the first sampling was 47 days. We found that the patients having received vaccination had a higher antibody level at admission than

unvaccinated patients, and the overall median of SARS-COV-2 IgM and SARS-COV-2 IgG were 0.19 and 1.13, respectively (Figure 3). The median levels of SARS-COV-2 IgM and SARS-COV-2 IgG in unvaccinated patients were 0.05 and 0.10, respectively; while in fully vaccinated patients, the median levels were 0.30 and 4.77, respectively (Table 2). Furthermore, we drew the scatter plot of the relationship between the antibody level and duration of viral shedding (Figure 3 (C), (D)) and found that with the increase of antibody concentration, the duration of viral shedding turned shorter. To explore how the dynamic antibody concentration influenced the patient's prolonged viral shedding duration, we made further analysis using the JM with repeated measurement antibody data.

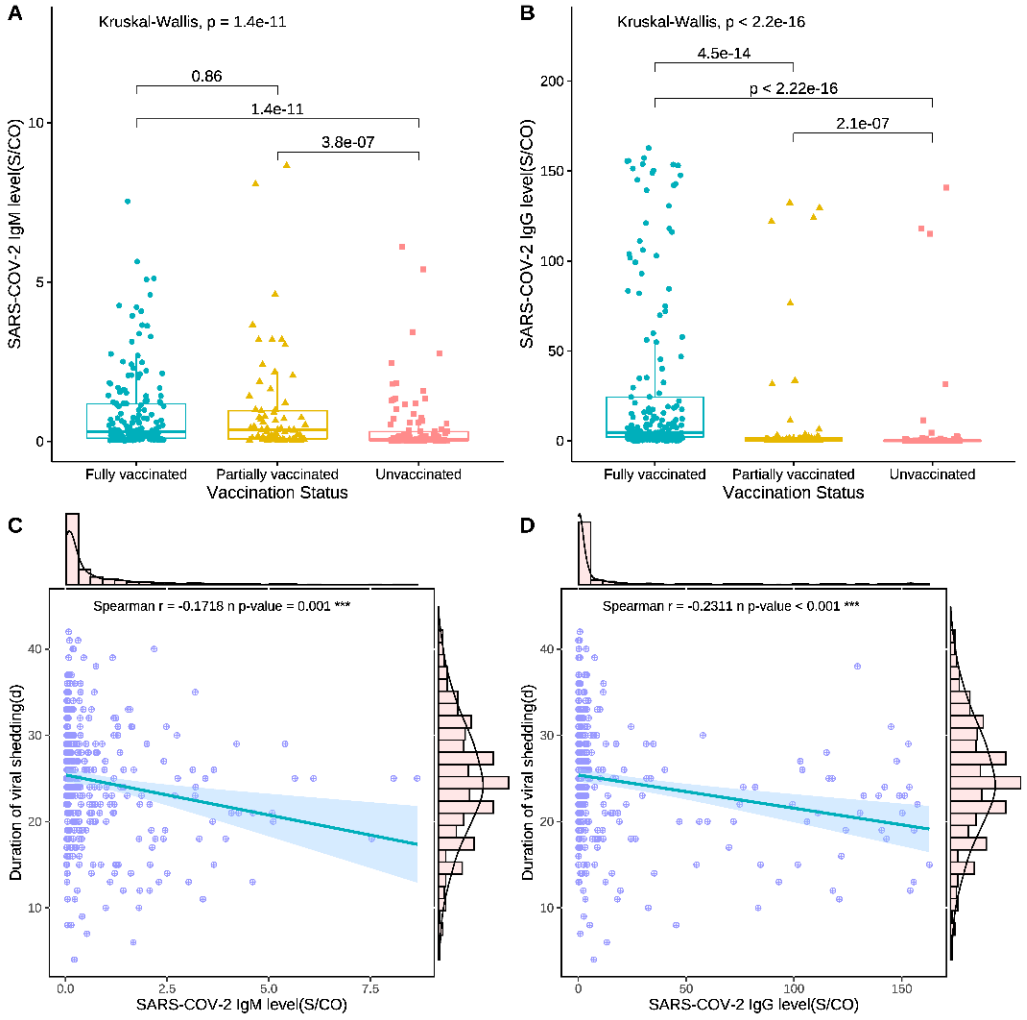

Figure 3. Correlation analysis of vaccination status, antibody concentration and duration of viral shedding. **A.** Boxplot of the relationship between SARS-COV-2 IgM level(S/CO) and vaccine status (fully vaccinated, partially vaccinated, and unvaccinated); **B.** Boxplot of the relationship between SARS-COV-2 IgG level(S/CO) and vaccine status (fully vaccinated, partially vaccinated, and unvaccinated); **C.** Marginal Density Scatter Plot of the relationship between SARS-COV-2 IgM level(S/CO) and duration of viral shedding (d); **D.** Marginal Density Scatter Plot of the relationship between SARS-COV-2 IgG level(S/CO) and duration of viral shedding (d).

Table 2. Distribution of antibody in different vaccination status.

	Vaccination status			
Type of antibody	Overall	fully vaccinated	partially vaccinated	unvaccinated
SARS-COV-2 IgM (median [IQR])	0.19 [0.06, 0.75]	0.30 [0.10, 1.17]	0.36 [0.08, 0.96]	0.05 [0.03, 0.31]
SARS-COV-2 IgG (median [IQR])	1.13 [0.15, 7.61]	4.77 [2.24, 24.41]	0.43 [0.15, 1.62]	0.10 [0.05, 0.25]

Abbreviations: SARS-COV-2 IgM, Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin M; SARS-COV-2 IgG, Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin G.

3.3. JM Analysis

Figure 4 provides sample subject-specific longitudinal traces for log SARS-COV-2 IgM (S/CO) in patients with and without endpoints. The figure clearly depicts the complexity of the data and the flatter SARS-COV-2 IgM (S/CO) levels in patients with 21-day viral shedding. The fitted model takes into account the relevant random intercept and slope of the model. The results of the linear mixed-effects model showed the longitudinal variation of logSARS-COV-2 IgM (S/CO) values, with a parameter estimate of 0.193 and a standard error of 0.017 (Table 3). A significant increasing trend was observed in Log (SARS-COV-2 IgM (S/CO)) over time. Then, a Cox model was fitted, with gender as an interdependent variable, and the risk function of viral shedding (or not) within 21 days was modeled as the outcome variable. The parameter estimates of the model and their standard errors are given in Table 4. Figure 5 shows the Kaplan-Meier of the probability of survival of viral shedding between the different genders. Finally, the joint model output showed that SARS-COV-2 IgM (S/CO) level was strongly associated with the risk of a composite event at the 95% confidence level (Table 5). A doubling of SARS-COV-2 IgM (S/CO) level was associated with a 1.38-fold[1] (95% CI: [1.16,1.72]) increase in the risk of viral non-shedding.

Table 3. Liner mixed model fixed parameter estimates.

Effects	Parameter	Estimate	Std Err	*P* value
Log(SARS-COV-2) IgM (S/CO))	Intercept	-1.405	0.082	< 0.001
	days	0.193	0.017	< 0.001

Abbreviations: SARS-COV-2 IgM, Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin M

[1] The difference on the logarithmic scale of IgM is 0.693, which corresponds to a ratio of 2 on the original scale, so exp(0.693 Assoct) gives the corresponding hazard ratio for doubling of IgM.

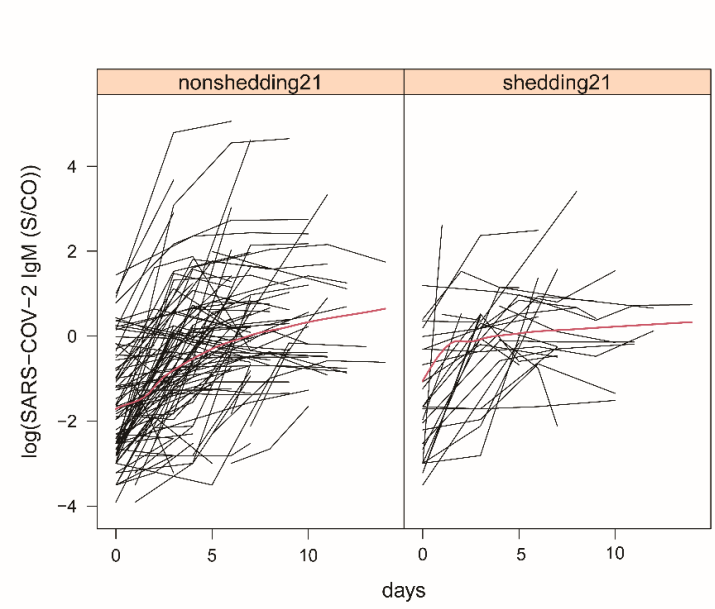

Figure 4. Subject-specific log SARS-COV-2 IgM (S/CO) longitudinal trajectories in patients with viral shedding and viral non-shedding. Red line indicates smoother.

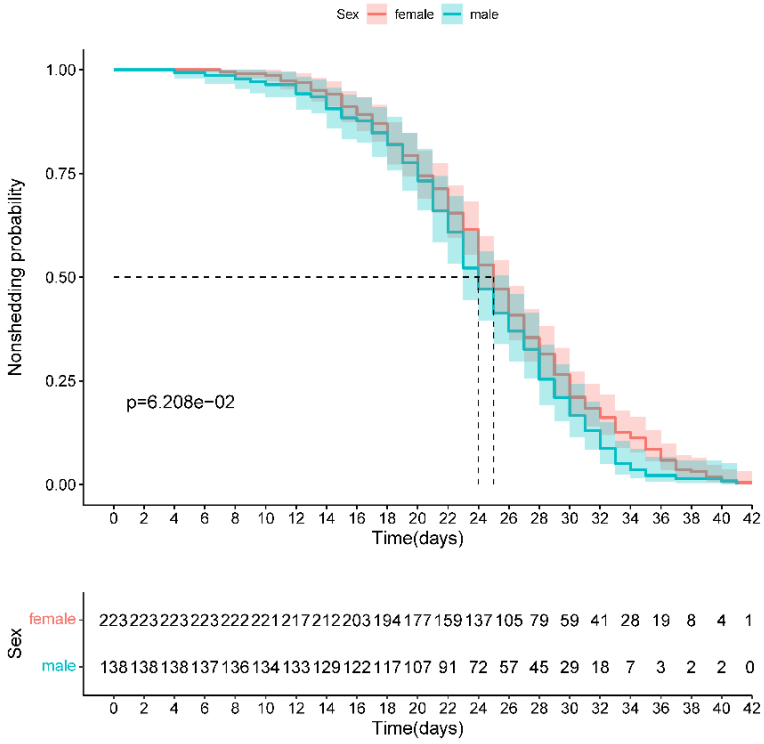

Figure 5. Kaplan-Meier estimates of 21-day viral shedding probability by gender.

Table 4. Cox proportional hazards model parameter estimates.

Parameter	Estimate	exp(coef)	Std Err	*P* value
Sex	0.205	1.228	0.1093	0.0608

Table 5. Joint model parameter estimates.

Parameter	Estimate	Std Err	*P* value
Sex	0.274	0.005	0.056
Assoct	0.430	0.039	<0.001

3.4. Dynamic prediction

Based on the joint model, we made dynamic prediction about the survival outcome of a randomly selected individual. More formally, based on the joint model, we were interested in deriving a probability prediction of viral shedding in a subject who provided a set of longitudinal measurements. With the help of the survivfitJM () and predict () functions in the JMbayes package, we dynamically predicted the time to viral shedding in Patient 361 based on the values of longitudinal changes in SARS-COV-2 IgM (S/CO) antibody level (Figure 6).

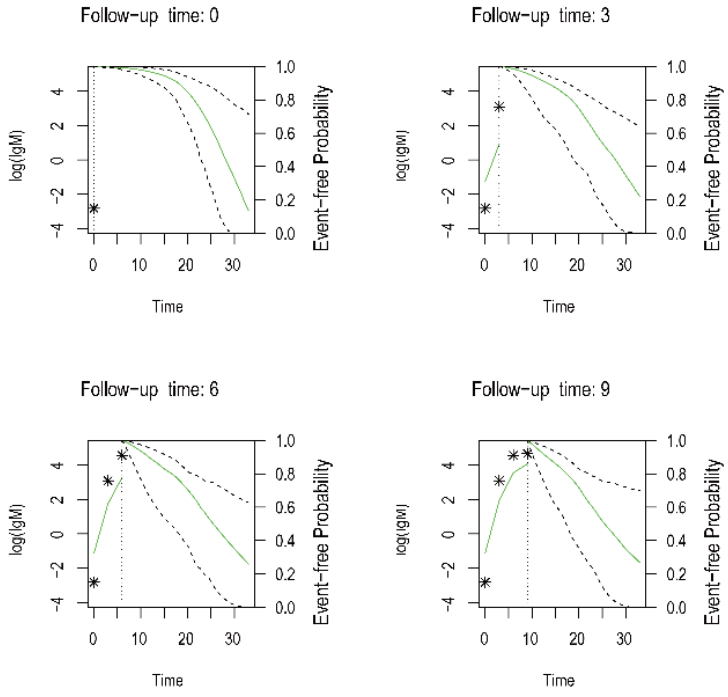

Figure 6. The dynamic survival probability of Patient 361 during follow-up under the joint model. The vertical dashed line indicates the time point of the last SARS-COV-2 IgM (S/CO) antibody test. The left side of the vertical line depicts the fitted longitudinal trajectory. The solid line to the right of the vertical line indicates the median estimate of the dynamic survival probability, and the dashed line corresponds to the point-by-point 95% confidence interval.

We observed that Patient 361 had a low baseline SARS-COV-2 IgM (S/CO) antibody level at admission and her probability of no shedding virus within 21 days was high. But her longitudinal profile showed a sharp increase in IgM antibody level, and accordingly, her probability of shedding virus within 21 days increased.

4. Discussion

In this study, we established a joint model, which took full advantage of repeated measurements, to explore the factors contributing to the prolonged viral shedding. We found little connection between the duration of viral shedding and some basic variables varying with time, such as routine blood indicators, though they had been measured at many time points with slight fluctuation. Through correlation analysis, patients having received vaccination were found to have higher antibody levels, and at the same time, baseline information showed that prolonged viral shedding was related to a low antibody level. Using the linear mixed-effects model, we found that the concentration of SASRS-COV-2 IgM (S/CO) varied with time obviously. Through the Cox proportional hazards model, difference was found in the length of viral shedding between the two genders. By combining the results of the linear mixed-effects model into the Cox model, the joint model output showed that SARS-COV-2 IgM (S/CO) level was strongly associated with the risk of a composite event at the 95% confidence level, with a doubling of SARS-COV-2 IgM (S/CO) level and an increased risk of 1.38-fold (95% CI: [1.16,1.72]). A study has found that COVID-19 patients with positive anti-SARS-CoV-2 IgM results have a short duration of viral shedding [24], which is consistent with the finding in this study. Our study is the first to investigate the correlation between SARS-COV-2 IgM (S/CO) and Delta variant-infected patients using datasets with repeated measurements and time-to-event outcomes.

SARS-CoV-2 spike binds to its receptor ACE2 through its receptor-binding domain (RBD) to enter human cells [25]. High levels of IgM, and IgG anti-SARS-CoV-2 spike protein and RBD binding titer were found in volunteers after the second vaccine injection [26]. Besides, IgM plays a pivotal role in both humoral and mucosal immunity and it is a mucosal antibody that constitutes the first line of defense against mucosal pathogens [27]. Moreover, IgM antibodies that contain neutralizing antibodies directed against different epitopes of the Spike glycoprotein [28,29]. When infected by SARS-CoV-2, neutralizing antibodies recognize multiple regions within the spike glycoprotein, primarily in but not limited to the RBD, and inhibit viral infectivity through multiple mechanisms, including blocking the initial spike binding to ACE2 [29,30]. Ku et al. [31] engineered an IgM neutralizing antibody, which offered broad protection from SARS-CoV-2 variants. Vaccines can reduce the COVID-19-related hospitalization and death, as well as the asymptomatic SARS-CoV-2 infection [32]. A study has showed that infections occurring 12 days or longer after vaccination can significantly reduce viral loads, potentially affecting viral shedding and contagiousness [33]. Also, Chia et al. 9 have found a faster decrease of viral load and stronger boosting of anti-spike antibodies in vaccinated patients with Delta variants compared to those unvaccinated.

There are some advantages in this research. We made full use of the patients' longitudinal dada, adding to the credibility of the results. Using the joint model we established, the dynamic prediction was made about the survival outcome of a given individual, providing more accurate anticipations to health workers. Also, we confirmed that patients having been vaccinated had a higher antibody level, thus accelerating viral shedding. However, there are some limitations in our study: We failed to find the impact of SARS-CoV-2 IgG on viral shedding, which might be attributed to insufficient sample

size. And we guess that the longitudinal data of IgG may exert impacts on severe patients, which needs further study. Moreover, with the emergence of the new variant of COVID-19, the Omicron variant has spread widely in China, but this study on the relationship between antibodies and viral shedding can still provide certain methods and ideas for similar research on different variants in the future.

5. Conclusions

By making full use of the patients' longitudinal records, we established the joint model, suggesting that higher antibody level in vaccinated patients, along with the presence of high-level SARS-COV-2 IgM antibodies in the serum, can accelerate viral shedding. This model can maximize the use of individual repeated data, explore the influencing factors of virus shedding, and provide certain ideas for relevant personnel to formulate prevention and treatment strategies for SARS-CoV-2.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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