의학박사 학위논문

면역저하 COVID-19 감염환자의 바이러스 배출 동역학 및 격리 해제를 위한 신속항원 검사의 유용성

Virological Characteristics and the Rapid Antigen Test as De-isolation Criteria in Immunocompromised Patients with COVID-19 : A Prospective Cohort Study

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2023년 5월

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Abstract in English

Objectives: Patients with moderate to severe immunodeficiency who are infected with SARS-CoV-2 are known to shed the virus for an extended period. The Centers for Disease Control and Prevention (CDC) recommends that those patients should be isolated at least 20 days, and undergo serial testing with consultation with as infectious specialist in order to de-isolate. However, there are limited data supporting current CDC guidelines for the isolation period in the moderate to severely immunocompromised patients with COVID-19 are limited.

Methods: Adult COVID-19 patients who underwent solid organ transplantation (SOT) or received active chemotherapy against hematologic malignancy were enrolled and instructed to provide weekly respiratory samples. Assessments of genomic and subgenomic viral copy numbers were conducted to collected samples. Samples with positive genomic real-time PCR results underwent virus culture and rapid antigen testing (RAT). All-cause mortality, according to shedding period of SARS-CoV-2, was also analyzed. In order to determine the duration of viable virus shedding and its association with clinical outcomes, the manifestations of pneumonia were classified into three forms: delayed, relapsed, and protracted. The association between each form and the duration of virus shedding was evaluated. Finally, the impact of prolonged virus shedding on patient prognosis was assessed.

Results: A total of 65 patients (40 with hematologic malignancy and 25 with solid organ transplantation) were enrolled. The median duration of viable virus shedding was 4 weeks (interquantile range [IQR] 3 – 7). Multivariable analysis revealed that B-cell depletion (hazard ratio [HR] 4.76, 95% Confidence Interval [CI] 1.11 – 20.00) was associated with prolonged viral shedding, and COVID-19 vaccination (≥ 3 doses) was negatively associated with prolonged viral shedding (HR 0.22, 95% CI 0.06 – 0.77). The sensitivity, specificity,

positive predictive value, and negative predictive value of RAT for viable virus shedding were 79%, 76%, 74%, and 81%, respectively. The negative predictive value of RAT was only 48% (95% CI 33-65) in the samples from those with onset ≤ 20 days, but it was as high as 92% (95% 85-96) in the samples from those with onset > 20 days. On the other hand, relapsed or delayed pneumonia significantly extended the duration of virus shedding (median duration of 8 weeks versus median duration of 4 weeks), whereas patients with protracted pneumonia did not exhibit a significantly prolonged virus shedding period. The survival analysis showed that mortality rate of patients was significantly higher in the group with a virus shedding duration of 6 weeks or longer (p=0.043), while no significant difference was observed for durations below 6 weeks.

Conclusions: About half of immunocompromised COVID-19 patients shed viable virus for ≥ 4 weeks from the diagnosis, and virus shedding was prolonged especially in unvaccinated patients with B-cell depleting therapy treatment. RAT beyond 20 days in immunocompromised patients had a relatively high negative predictive value for viable virus shedding, which is supporting current CDC's recommendation of de-isolation of moderate to severely immunocompromised patients with COVID-19.

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Introduction

The Omicron variants of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) are associated with lower mortality and severity than previous variants of concern [1]. Nevertheless, immunocompromised patients are still one of the most vulnerable groups of coronavirus disease-2019 (COVID-19) patients, with high morbidity and mortality [2, 3]. This high mortality of COVID-19 in immunocompromised patients mainly arises from two reasons; one is enhanced virulence due to COVID-19 itself, and another one is delayed therapy for underlying illness during the prolonged isolation. Thus, optimizing the isolation period of immunocompromised patients with COVID-19 is important not only for the mean of infection control, but also for the improving patient outcome.

Several studies reported that the protracted course of COVID-19 in immunocompromised patients is due to an impaired immune response against SARS-CoV-2 infection and is associated with prolonged virus shedding [4, 5]. However, those reports only included small case series. Furthermore, this manifestation of COVID-19 in immunocompromised patients is the matter of debate whether is due to enhanced viral virulence or due to unregulated immune response [4, 6]. In immunocompetent patients with COVID-19 pneumonia, current guidelines recommend administration of immune-modulatory therapeutics, such as steroid, baricitinib, or tocilizumab [7]. However, these approaches are not yet been evaluated in immunocompromised setting, and there is concern about administration of immune-modulatory therapeutics may harm to those with active viral shedding, such as immunocompromised COVID-19 patients [6]. Therefore, understanding of pathophysiology of protracted or delayed manifestation of COVID-19 pneumonia in immunocompromised patients is important.

On the other hands, immunocompromised patients with COVID-19 shed viable virus for prolonged periods up to several months [5, 8, 9], while immunocompetent patients shed viable virus for 7 to 10 days [10, 11]. While the Centers for Disease Control and Prevention (CDC) recommends an isolation period of at least 20 days for moderately or severely immunocompromised patients with COVID-19, and ending isolation in conjunction with serial testing and consultation with an infectious disease specialist [12], these recommendations are based on limited data.

The golden standard of determining the isolation period of patients with COVID-19 is virus culture [13]. However, this approach is not practical, as virus culture is not economic and time-consuming [14]. Several alternative approaches have been proposed to overcome the limitations of virus culture, including prediction model, real-time PCR (both genomic and subgenomic), and rapid antigen tests [14-17]. In previous study, a prediction model for viable virus shedding in patients with COVID-19 was proposed [14]. However, this model has limitations of small numbers of immunocompromised patients, only included patients infected to pre-omicron strain of COVID-19, and not externally validated [14]. The genomic real-time PCR has an advantage in excellent accessibility, but also has limitations that even healthy convalescent patients can shed RNA for long period, poor external validity, and no agreement of infectious threshold [14, 15, 18, 19]. Especially, Jeong et al. demonstrated that small alteration of infectious threshold of genomic RNA PCR can lead to a significant difference of isolation policy [20]. The subgenomic RNA PCR is promising technique for prediction of viable virus shedding, while its performance is still a matter of debate [17, 21, 22].

The performance of rapid antigen tests for ending isolation of immunocompetent patients with COVID-19 was evaluated in several previous studies [16, 23]. Those studies indicated

that acceptable negative predictive value of rapid antigen test when test was performed in the period with low expectation of culture positivity, usually later than day 5 [23]. These findings suggest possibility of using rapid antigen test as evidence of ending isolation in immunocompromised setting, while this assumption is never been evaluated. In previous study, Jeong et al. found that less than 5% of risk of premature ending of isolation is not achievable in single-test strategy, and this assumption is base of current double-test strategy [20]. Therefore, I assumed that acceptable risk of premature ending is less than 5%.

This study builds on an earlier publication [24] in which I evaluated viable virus shedding for a longer period in a larger sample of immunocompromised subjects by longitudinal dense respiratory sampling, and identified factors associated with prolonged shedding. In the present work, I also evaluate the performance of the rapid antigen test for predicting virus culture results.

Methods

1. Study population

From February 1, 2022 to September 1, 2022, I prospectively enrolled immunocompromised patients with COVID-19 who were admitted to Asan Medical Center, a 2700-bed hospital located in Seoul, South Korea and received active chemotherapy against hematologic malignancy or underwent solid organ transplantation. COVID-19 was diagnosed by RT-PCR. Adult patients (age > 18 years) within 12 weeks from diagnosis of COVID-19 were included, while patients with a known history of prior COVID-19 were excluded. In Asan Medical Center, PCR tests were administered in three situations; when patients had close contact with

COVID-19 patients, as a screening test for admission, and to diagnose patients who were experiencing symptoms consistent with COVID-19.

Weekly dense respiratory samples were collected until 12 weeks after diagnosis or discharge, and sample collection was extended ---in conjunction with an Infectious Diseases consultation--- if clinical findings or repeated RT-PCR results were compatible with COVID-19 or suggestive of viable virus shedding. Saliva was collected for the dense respiratory sampling, but nasopharyngeal (NP) swabs were used instead when patients found collecting saliva difficult due to clinical deterioration. Viral copy numbers were evaluated by means of genomic RNA PCR and subgenomic RNA PCR, and samples with positive genomic RNA PCR results, defined as > 2.6 viral copies/mL (95% limit of detection), underwent virus culture and rapid antigen testing. All patients gave written informed consent, and the institutional review board of Asan Medical Center approved the study design (IRB-2022-1054).

2. Definitions

Patients with COVID-19 pneumonia were divided into three categories: protracted pneumonia, relapsed pneumonia, and delayed pneumonia (Figure 1). Protracted pneumonia was defined as COVID-19 pneumonia with no improvements in radiologic finding and clinical features after 2 weeks of treatment; Relapsed pneumonia was defined as development of pneumonia that was not fully explained by any other cause and shares radiologic findings with COVID-19 pneumonia after resolution of the clinical features. Delayed pneumonia was defined as development of COVID-19 pneumonia two weeks after diagnosis in patients with no previous evidence of pneumonia.

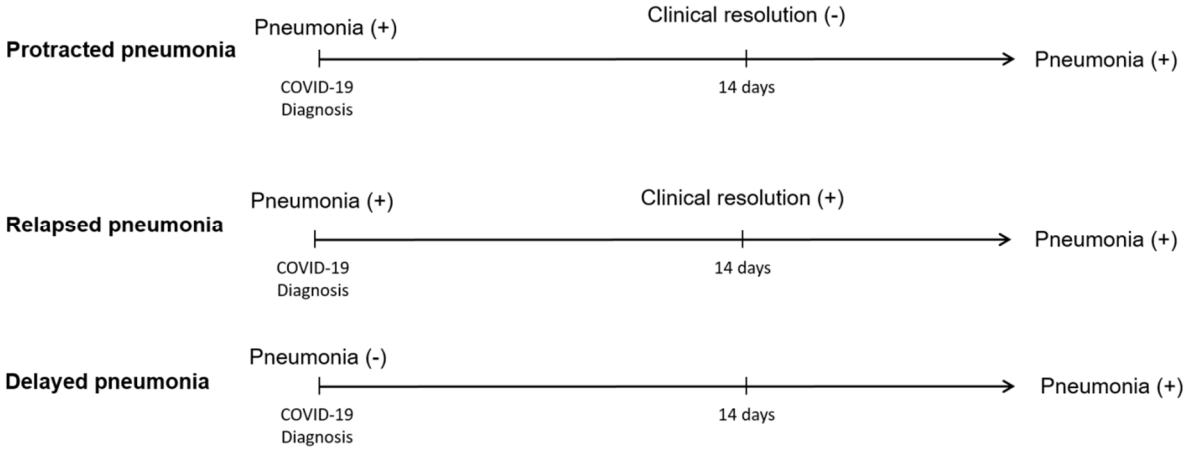


Figure 1. Definition of protracted, relapsed, and delayed pneumonia

Immunocompromised status was defined according to the definition in current CDC guidelines[25]. B-cell depletion status was defined as any CD-19- or CD-20-specific antibody within the 6 months before diagnosis of COVID-19 or 1 month after diagnosis. T-cell depletion status were defined as administration of any CD-3-specific antibody agent in the aforementioned period. Severity of COVID-19 was defined according to the U.S. National Institutes of Health [26].

Antiviral agents against COVID-19 were defined as administration of any of the following drugs; remdesivir, molnupiravir, and nirmatrelvir/ritonavir. “Early” antiviral therapy was defined as administration of agent within one week of COVID-19 diagnosis, while “late” antiviral therapy was defined as use of an agent more than a week after COVID-19 diagnosis.

3. Detection of genomic and subgenomic RNAs

Viral RNA was extracted from respiratory specimens using a QIAamp viral RNA Mini kit (Qiagen Inc., Hilden, Germany) according to the manufacturer’s instructions. PCR reaction mixtures for detecting genomic RNA (20 μL) contained 0.1 μL of 200× enzyme mix, 4 μL of 5× master mix (LightCycler Multiplex RNA Virus Master, Roche, Basel, Switzerland), 500 nM each of S and N gene primers, 200 nM of S and 250 nM of N gene probes, 250 nM of internal control primers and 125 nM of internal control probes as described previously [12]. For subgenomic RNA, reaction mixtures contained 0.1 μL of 200× enzyme mix, 4 μL of 5× master mix, 1000 nm of leader primer, 500 nM each of S and N gene reverse primers, 250 nM of S and N gene probes, and internal control primers and probes as described previously [12]. Each mixture contained 5 μL of test RNA or in vitro-synthesized control RNA.

Amplification was performed with a LightCycler 96 system (Roche) in the following conditions: reverse transcription at 50 for 10 min, initial denaturation at 95 for 5 min, 45 cycles of 2-step amplification, denaturation at 95 for 10 s, annealing and elongation at 60 for 30 s, and final extension at 60 for 5 min. Calibration curves were generated using serial dilutions from 10^7 to 5 copies/μL of synthetic control RNA assayed in six independent sets of reactions. The detection limit of the assay was 5 copies/reaction (2.6 log copies/ml of specimen), and viral copy numbers were determined by plotting Ct values against log copies/reaction (Table 1) [18]. Decisions concerning virus positivity, and measurements of viral loads, were based on the N gene.

Table 1. Relationship between Viral copy number and Ct value.

Viral copy number (log copies/mL)	Ct value
8	17.45
7	20.78
6	24.12
5	27.45
4	30.79
3	34.12

4. Identification of SARS-CoV-2 variants

Viral RNA was extracted from respiratory specimens with a QIAamp viral RNA Mini kit (Qiagen). SARS-CoV-2 Omicron variants were identified by multiplex real-time RT-PCR. PCR reaction mixtures (20 μL) included 10 μL of 2X PCR master mix, 5 μL of primer/probe mix, and 5 μL of extracted RNA. PCR amplification was performed with an Applied Biosystems 7500 Fast Dx Real-time PCR instrument (Applied Biosystems. Waltham, MA, USA) in the following conditions: reverse transcription at 50 for 15 min, initial denaturation at 95 for 5 min, 5 cycles of pre-amplification, denaturation at 95 for 10 s, annealing and elongation at 60 for 30 s, 40 cycles of amplification, denaturation at 95 for 10 s and annealing and elongation at 60 for 30 s. Omicron BA.1 and 2 were identified with a PowerChek SARS-CoV-2 S-gene mutation detection kit ver. 3.0 (Kogene Biotech, Seoul, South Korea). The Omicron BA.2 variant was defined as detection of the E484A, K417N, and N501Y mutations, and omicron BA.1 as detection of these three mutations plus T547K. Omicron sub-variants including BA.2.12.1, BA.2.3, BA.2.75, BA.4 or BA.5 were further distinguished with a PowerChek SARS-CoV-2 Omicron variants II real-time PCR kit (Kogene Biotech).

5. Virus culture and rapid antigen tests

Virus culture was performed on the samples positive for genomic RNA by RT-PCR. All procedures were performed in a Biosafety Level 3 (BL3) facility according to the laboratory biosafety guidelines of the Korea Disease Control and Prevention Agency. Briefly, pretreated and filtered samples were inoculated into wells of 24-well plates that had been seeded with Vero-E6 cells (1.5×10^5 cells/well) 24 hours earlier. The plates were

incubated for 1 hour at 37 with 5% CO_2. The medium in the wells was then replaced with Dulbecco's Modified Eagle Medium (DMEM) containing 2% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin (PS).

The cells were examined daily for cytopathic effects (CPE) for 7 days, after which levels of viral RNA in the cell supernatants were determined by Real-time PCR (rRT-PCR). The supernatants were also passaged into cells and tested by rRT-PCR to confirm viral replication, as previously described. Samples positive for CPE and viral RNA were defined as shedding infectious virus.

Samples positive for viral RNA were also assessed for virus shedding with a rapid lateral flow test using PCL COVID19 Ag Gold (PCL, Inc. Seoul, Korea). The procedures were performed according to the manufacturer's instructions, with some modifications. Briefly, 200 μl of each saliva samples was mixed with the same volume of buffer and filter cap was covered. The filtered mixture was applied to a test kit and the result was read after 10 min.

6. Statistical analysis

Categorical variables were analyzed using Fisher's exact test, and continuous variables were compared using the Wilcoxon rank-sum test. Survival analysis was used to estimate viable virus shedding periods, and the log-rank test was used to compare viable virus shedding times. Landmark survival analysis was applied to adjust immortal time bias. Due to concern about interval censoring, significant variables in a univariate survival analysis were re-examined with a semi-parametric proportional hazard model using bootstrapping. Patients who had never been culture-positive were treated as having been interval-censored assuming that they were culture-positive on the date of first diagnosis. A Cox's proportional hazard model, stratified for age and sex, was constructed to evaluate risk

factors for prolonged viral shedding. Variables were selected from the risk factors for prolonged viral shedding in previous studies and statistically significant factors in the univariate analysis [14, 27], and multivariable analysis was performed by backward selection. The Schoenfeld residuals test was carried out on the constructed model, and there were no violations of the proportional hazard. To assess the clinical performance of the RAT we compared its sensitivity, specificity, positive predictive value and negative value with virus culture. P-value < 0.05 in a two-tailed test was considered statistically significant. All statistical analysis was performed using R for statistics version 4.1.1, and R package ‘ggplot2’, ‘survival’, ‘survminer’, ‘binom’, ‘rms’ and ‘IcenReg’ were also used.

Results

1. Study population

During the study period, a total of 65 patients were enrolled (Table 2). Of these, 40 patients (62%) received active chemotherapy against hematologic malignancy, and 25 (39%) underwent solid organ transplantation. More than half the patients had received at least one dose of vaccine, while only 24 patients (37%) had completed a primary vaccine series. 42 patients (64%) were infected by SARS-CoV-2 BA2.3, followed by 11 (19%) with BA.4 or BA.5 and 10 with BA.1 (15%).

Fifty patients (78%) were asymptomatic or only had mild symptoms at diagnosis, while the remaining 15 patients had pneumonia. The symptoms failed to resolve in 9 (14%) of the 15 patients after 2 weeks of treatment. Twelve of the 51 patients (18%) developed delayed pneumonia, and 4 (6%) experienced relapsed pneumonia after clinical resolution of pneumonia.

Most of patients received COVID-19 specific treatment; 61 patients (94%) received remdesivir, 3 (5%) nirmatrelvir/ritonavir (paxlovid), 20 (30%) dexamethasone or an equivalent dose of steroid, 8 (12%) tocilizumab, and 11 (17%) baricitinib. Fourteen patients (22%) underwent B-cell depletion therapy; 8 received rituximab before COVID-19 diagnosis and 2 epocoritamab (bi-specific antibody). Three patients received rituximab before and after COVID-19 diagnosis, to treat antibody-mediated rejection of a kidney graft. T-cell depletion treatment was given to 6 patients (9%); 2 basiliximab before COVID-19 diagnosis, and the remaining 4 patients received anti-thymoglobulin after COVID-19 diagnosis.

Table 2. Baseline Characteristics of study participants

Variable	**Total** (n = 65)
Age, median years (IQR)	61 (49 – 66)
Male	45 (69)
Charlson's comorbidity index, median (IQR)	4 (3 – 5)
Hypertension	16 (25)
Diabetes mellitus	16 (25)
Solid cancer	6 (9)
Chronic kidney diseases	16 (25)
Chronic lung disease	2 (3)
Hematologic malignancy	40 (62)
Acute myeloid leukemia	11 (17)
Acute lymphoblastic leukemia	5 (8)
Myelodysplastic syndrome	5 (8)
Hodgkin lymphoma	1 (2)
Non-Hodgkin lymphoma	20 (31)
Solid organ transplantation	25 (39)
Kidney transplantation	12 (19)
Liver transplantation	9 (14)
Lung transplantation	2 (3)
Heart transplantation	2 (3)
Bone marrow transplantation (BMT)	18 (28)
Allo-BMT	6 (9)
Auto-BMT	12 (19)
Duration from transplantation, median days (IQR)	474 (179 – 3447)
B-cell depleting therapy	14 (22)
Before COVID-19[a]	10 (16)
After COVID-19[b]	7 (11)

T-cell depleting therapy[c]	6 (9)
Initial severity	
Asymptomatic	34 (53)
Mild	16 (25)
Moderate	8 (12)
Severe	6 (9)
Critical	1 (2)
Delayed onset or relapsed course pneumonia[d]	15 (23)
Delayed onset pneumonia[e]	12 (18)
Relapsed course pneumonia[f]	4 (6)
Protracted pneumonia	9 (14)
Vaccination status	
None	20 (31)
Partial (1- or 2-doses)	21 (32)
Completion of initial vaccine series (3-doses)	20 (31)
1st boosted (4-dose)	4 (6)
Time from last vaccination to infection, median days (IQR)[g]	120 (79 – 181)
Subvariant	
Omicron BA.1.	10 (15)
Omicron BA.2.3	42 (64)
Omicron BA.4 or BA.5	12 (19)
Undetermined	1 (2)
COVID-19-specific treatment	63 (97)
Remdesivir	61 (94)
Nirmatrelvir/ritonavir	3 (5)
Dexamethasone	20 (31)
Tocilizumab	8 (12)
Baricitinib	11 (17)

Culture positivity among PCR-positive samples	140/323 (43)
Saliva	128/276 (46)
Nasopharyngeal swab	12/46 (26)
Numbers of PCR-positive samples, median (IQR)	4 (3 – 6)
Date of first sample after diagnosis, median days (IQR)	4 (2 – 15)
30-day mortality	1 (2)
90-day mortality	11 (17)

NOTE. Data are presented as number of patients (%) unless otherwise indicated.
[a] 8 patients received rituximab; 2 patients received epocoritamab.
[b] All 7 patients received rituximab. 3 of those patients also received rituximab before COVID-19 diagnosis.
[c] 4 patients received anti-thymoglobulin after COVID-19 infection, while the remaining 2 patients received basiliximab before COVID-19 infection.
[d] 1 patient had relapsed pneumonia after resolution of delayed pneumonia.
[e] Delayed onset pneumonia was defined as development of COVID-19 pneumonia that was not fully explained by other causes at least 14 days after diagnosis in patients who were not initially diagnosed with COVID-19 pneumonia.
[f] Relapsed pneumonia was defined as re-occurrence of radiologic features and symptoms that confirmed COVID-19 pneumonia after resolution of the initial symptoms.
[g] 5 patients could not remember the date of their most recent COVID-19 vaccination.

2. Sample characteristics and duration of viral shedding

During the study period, a total of 482 samples were collected, of which 323 tested positive in real time RT-PCR (Table 1 and Figure 2A, 2B). Relationship between viral copy numbers and Ct values were shown in Table 2. Of the positive samples, 276 (85%) were of saliva, and the remaining 46 were from NP swabs (14%). Overall, 140 samples (43%) gave positive virus culture results, while 128 saliva samples (46%) and 12 NP swab samples (26%) were culture positive. Longitudinal changes of genomic, subgenomic RNA viral copy and culture positivity according to sample collection date are shown in Figure 1A and 1B. Weekly culture positivity and RAT positivity are shown in Figure 2C and 2D; they show that culture positivity and RAT positivity waned over time (P for trend < 0.001).

Figure 2A.

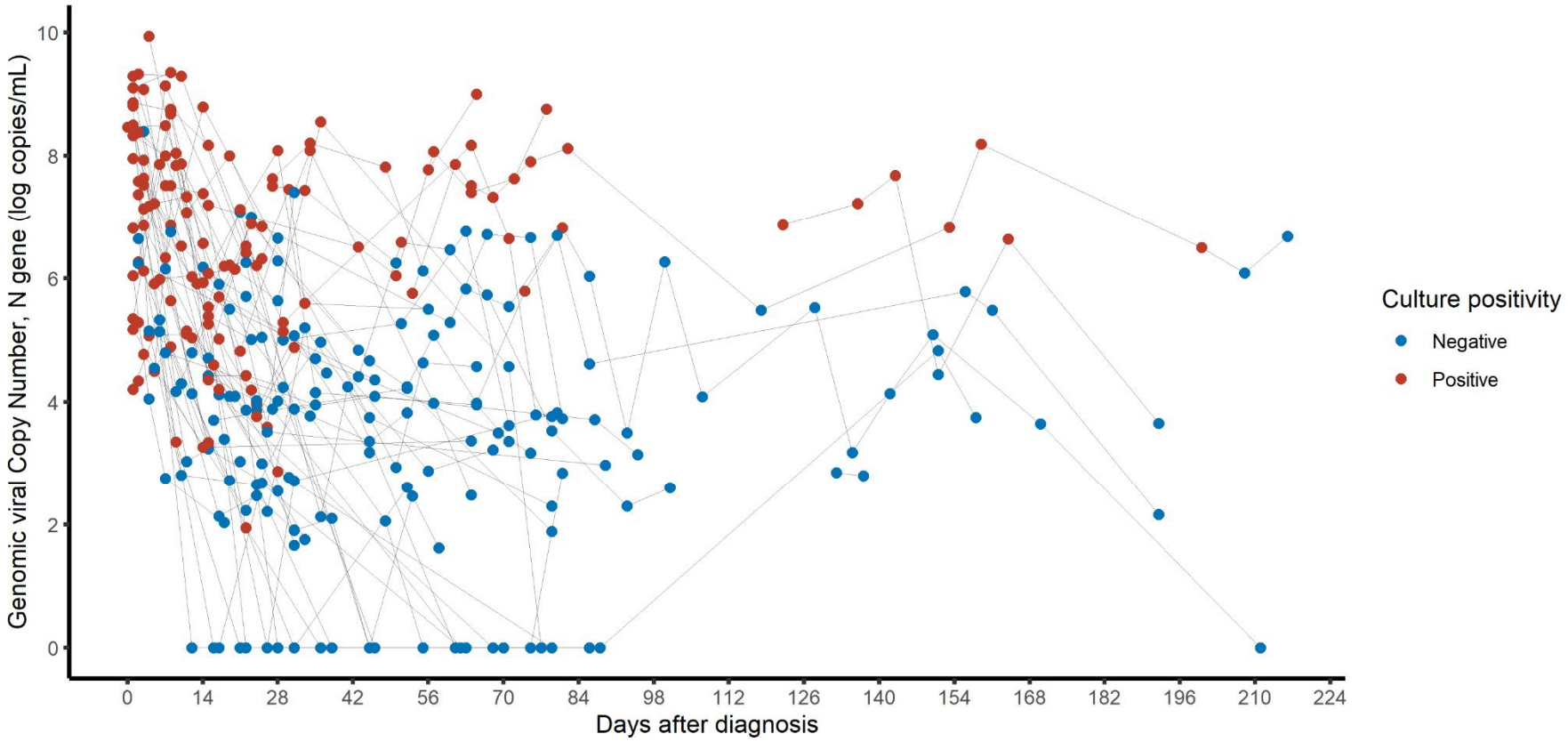

Genomic viral Copy Number, N gene (log copies/mL)
10
8
6
4
2
0
0
14
28
42
56
70
84
98
112
126
140
154
168
182
196
210
224
Days after diagnosis
Culture positivity
Negative
Positive

Figure 2B.

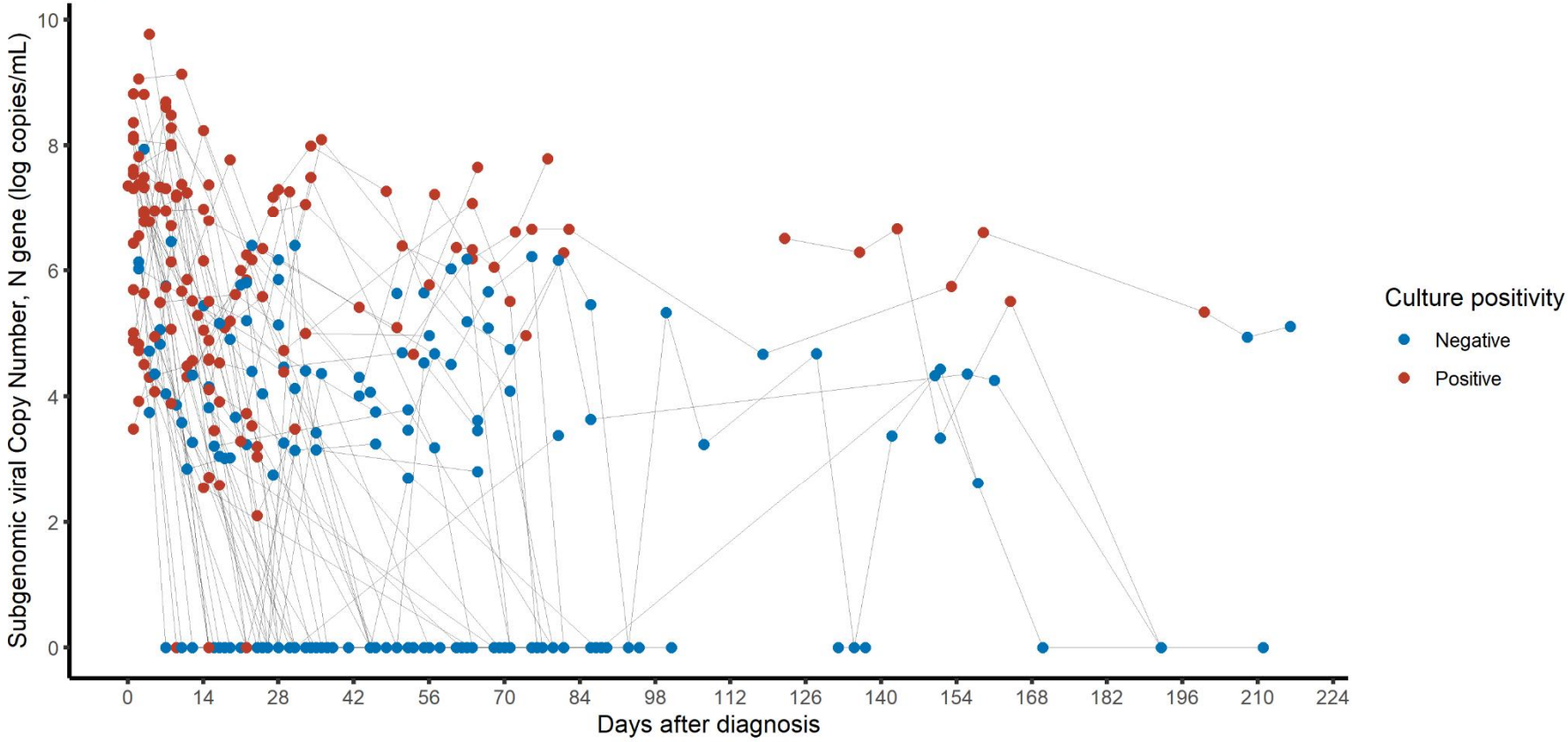
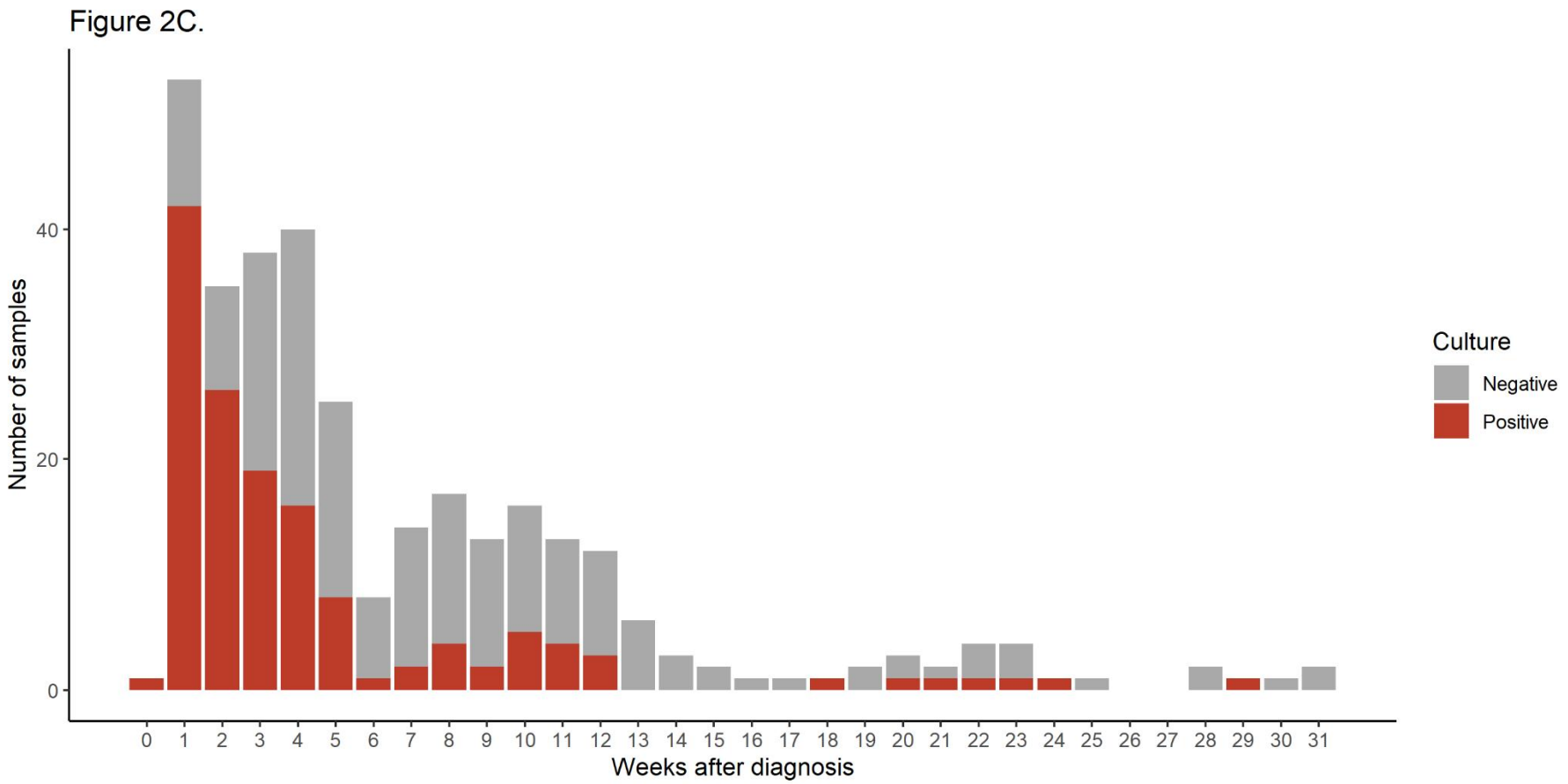

Subgenomic viral Copy Number, N gene (log copies/mL)
10
8
6
4
2
0
0
14
28
42
56
70
84
98
112
126
140
154
168
182
196
210
224
Days after diagnosis
Culture positivity
Negative
Positive

Figure 2C.


Number of samples
0
20
40
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Weeks after diagnosis
Culture
Negative
Positive

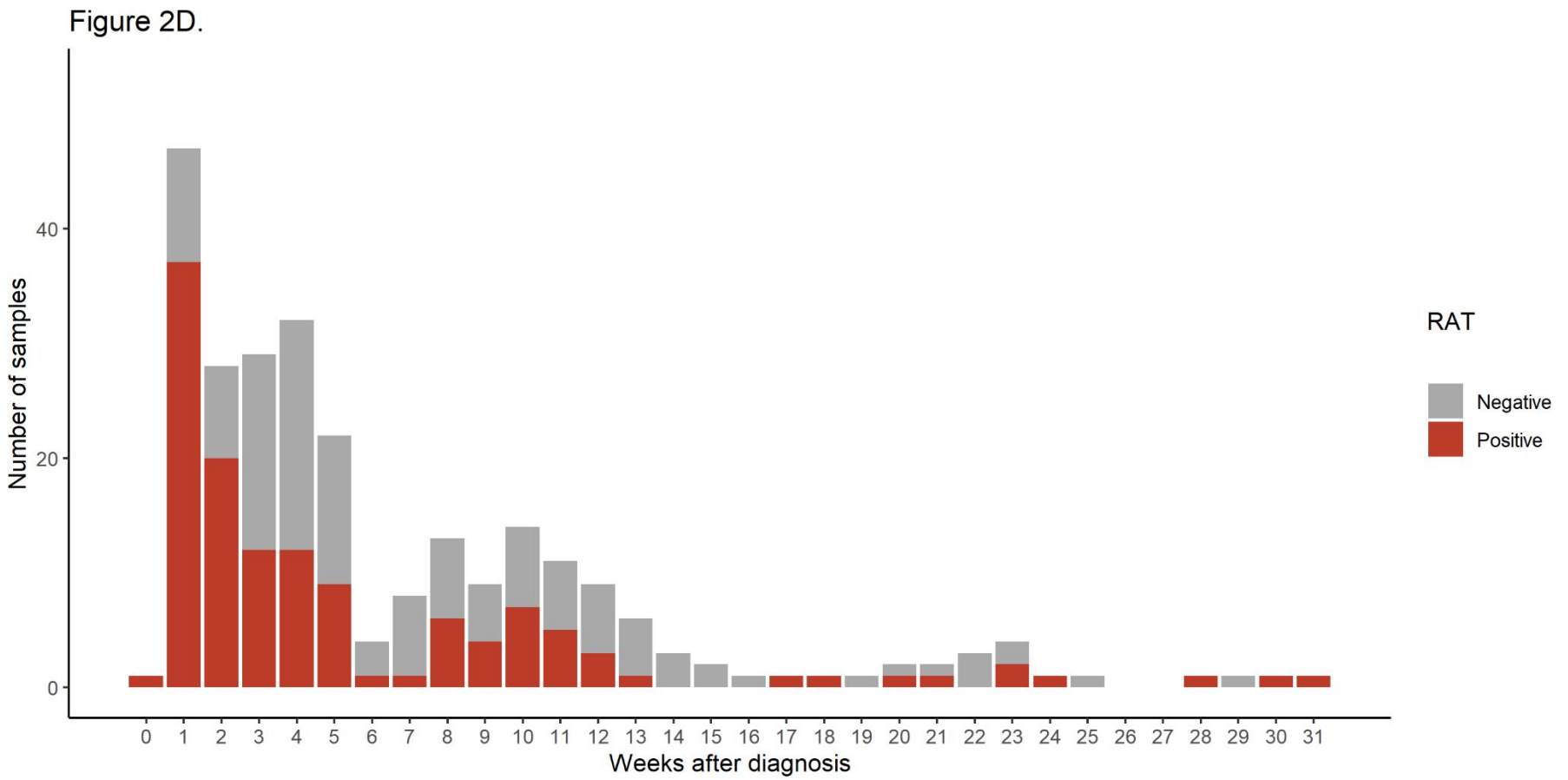


Figure 2. Longitudinal changes of RNA copy number and culture positivity in immunocompromised patients with COVID-19. 2A. Longitudinal changes of genomic viral copy number. **2B.** Longitudinal changes of subgenomic viral copy number. **2C.** Weekly culture positivity. **2D.** Weekly rapid antigen test (RAT) positivity.

Survival analysis revealed that immunocompromised patients shed viable virus for median 4 weeks (interquartile range [IQR] [3 – 7]), while subgenomic RNA shedding (median 7 weeks, IQR [4 – 12]) and genomic RNA shedding (median 12 weeks, IQR [6 – 31]) persisted for longer (Figure 3).

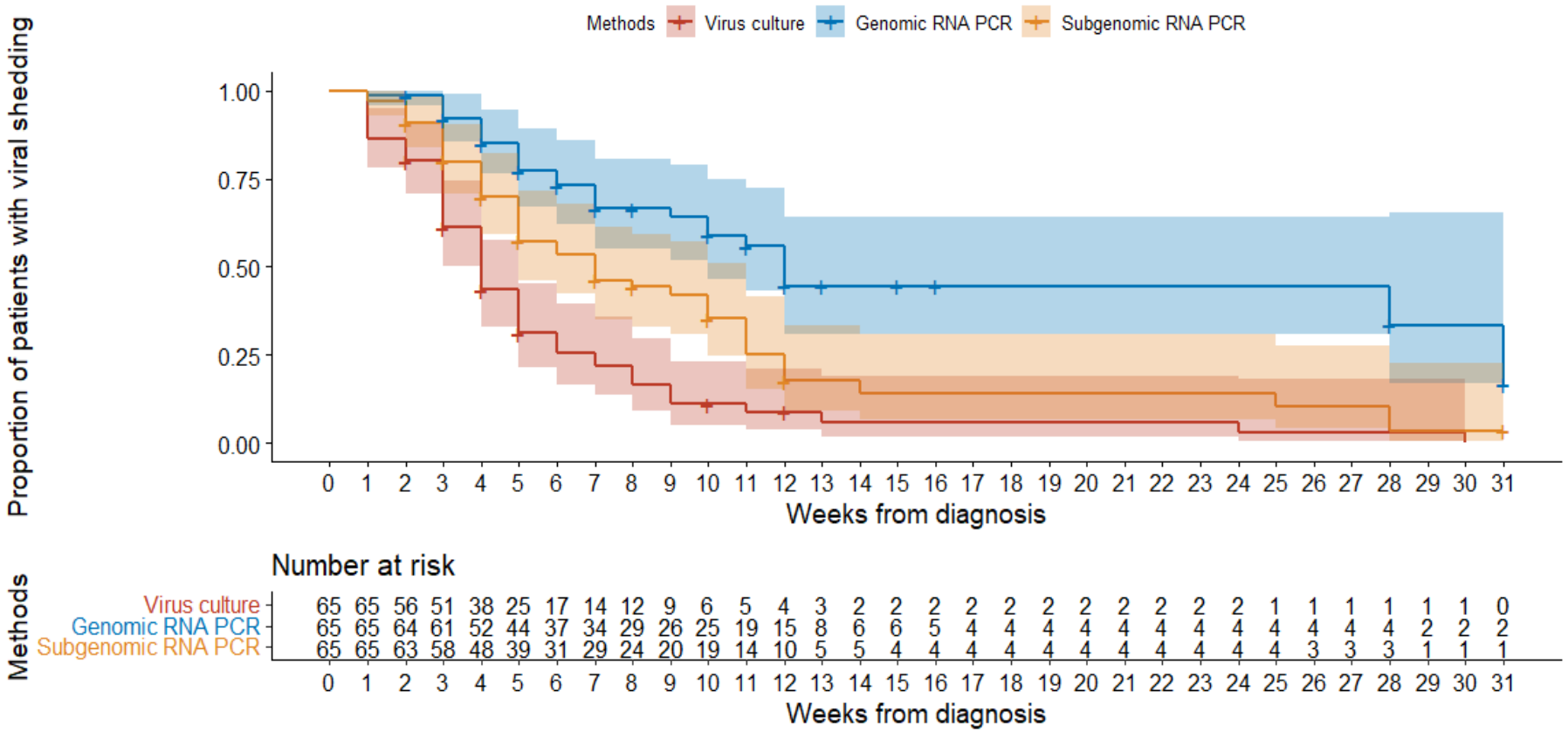


Figure 3. Kaplan-Meier curves for virus culture in immunocompromised patients with COVID-19. Red line indicates viable virus in terms of virus culture (median 4 weeks, IQR [3 – 7]), blue line indicates detection of genomic RNA (median 12 weeks, IQR [6 – 31]), and orange line indicates detection of subgenomic RNA (median 7 weeks, IQR [4 – 12]).

Various underlying conditions were examined to see whether they affected the viable virus shedding period (Figure 4). Receipt a B-cell depletion agent (median 8 weeks, IQR [5 – 9] vs. median 4 weeks, IQR [3 – 7], p = 0.004: Figure 4A), early antiviral administration (median 4 weeks, IQR [2 – 6] vs. median 5.5 weeks, IQR [3 – 24], p = 0.015: Figure 4C), development of delayed or relapsed pneumonia (median 8 weeks, IQR [4 – 11] vs. median 4 weeks, IQR [3 – 6], p = 0.005: Figure 4D), and exposure to active chemotherapy against hematologic malignancy (median 5 weeks vs. IQR [3 – 8] vs. median 4 weeks, IQR [3 – 4]) were significantly associated with prolonged viral shedding. These associations remained statistically significant even after adjusting the interval censoring (Figure 5). However, administration of T-cell depletion agent did not seem to affect the viable virus shedding period (median 4 weeks, IQR [3 – 7] vs. median 5 weeks, IQR [2 – 8], p = 0.95: Figure 4B).

Figure 4A.

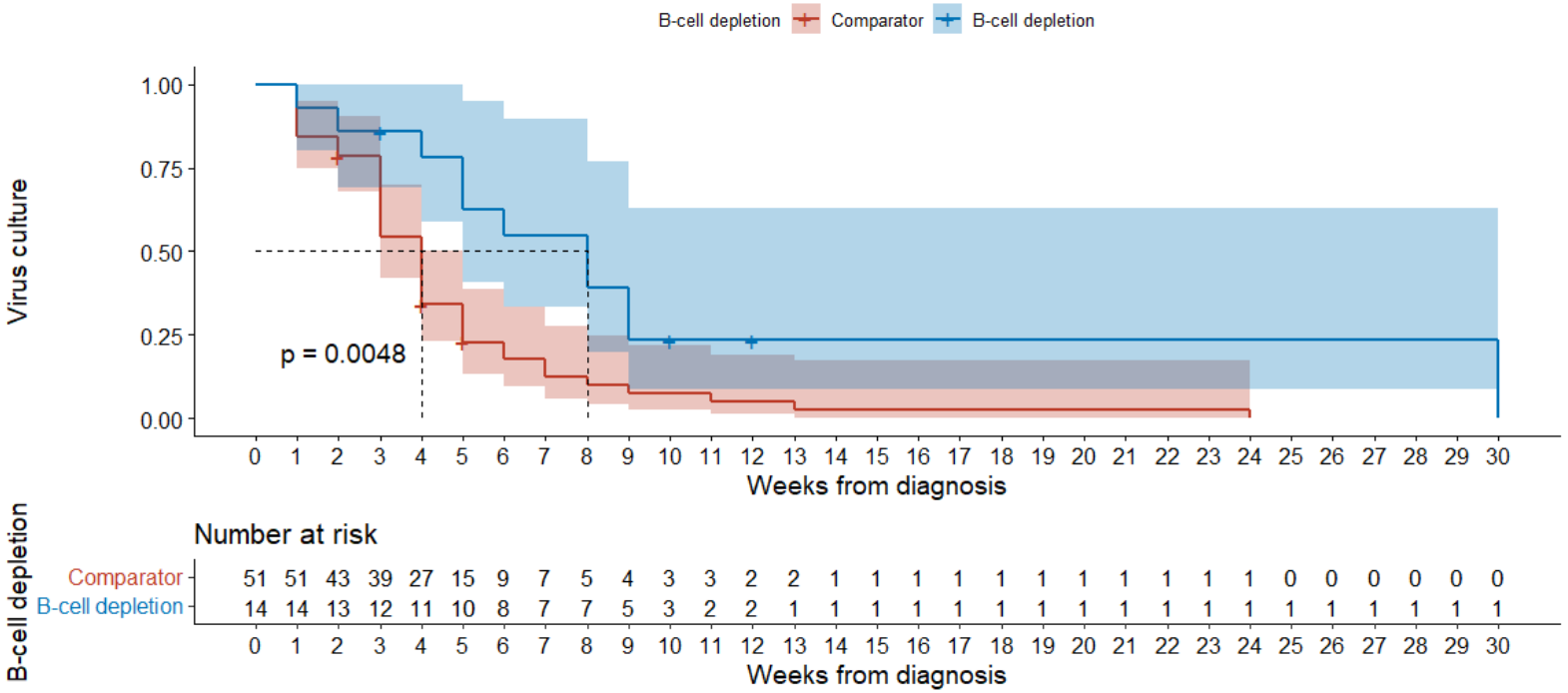

B-cell depletion
Comparator
B-cell depletion
Virus culture
1.00
0.75
0.50
0.25
0.00
p = 0.0048
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis
Number at risk
B-cell depletion
Comparator 51 51 43 39 27 15 9 7 5 4 3 3 2 2 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0
B-cell depletion 14 14 13 12 11 10 8 7 7 5 3 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis

Figure 4B.

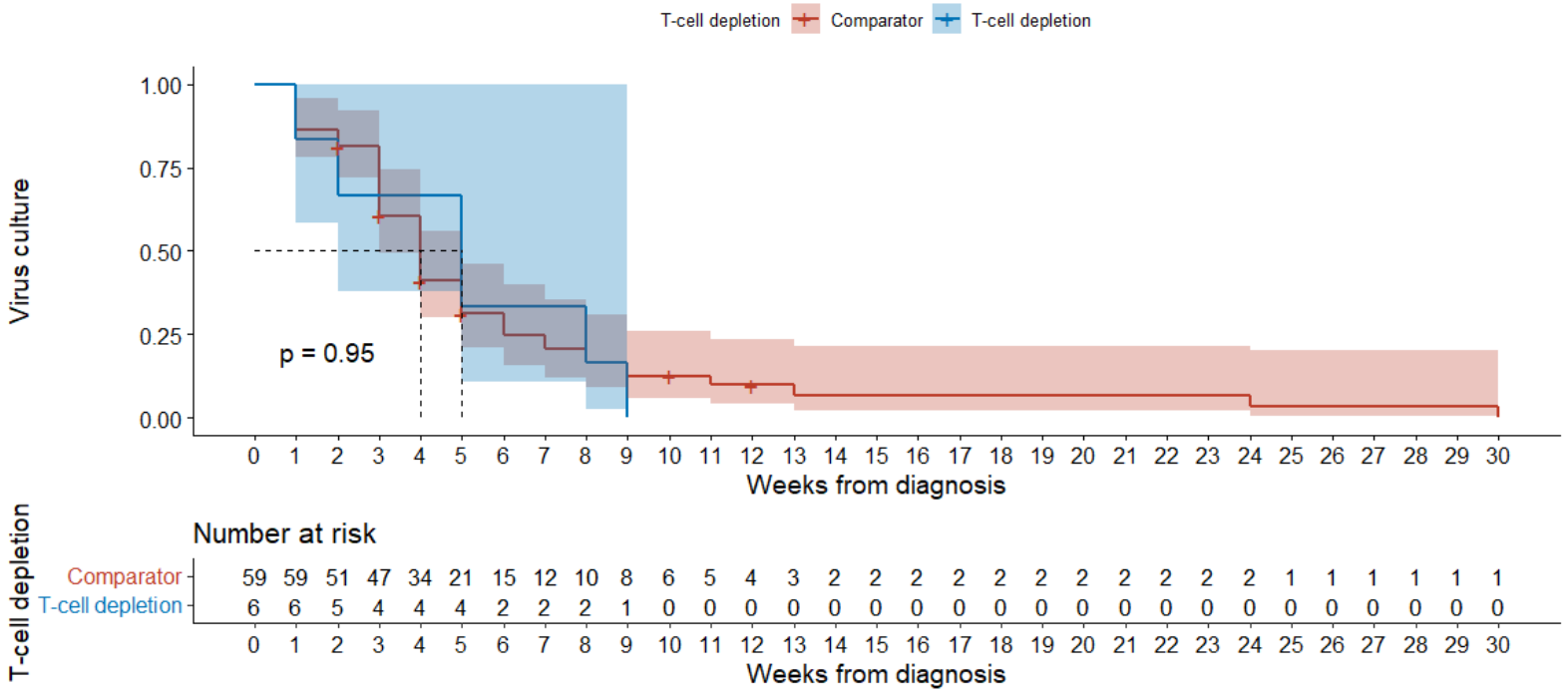

T-cell depletion
Comparator
T-cell depletion
Virus culture
1.00
0.75
0.50
0.25
0.00
p = 0.95
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis
T-cell depletion
Number at risk
Comparator 59 59 51 47 34 21 15 12 10 8 6 5 4 3 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1
T-cell depletion 6 6 5 4 4 4 2 2 2 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis

Figure 4C.

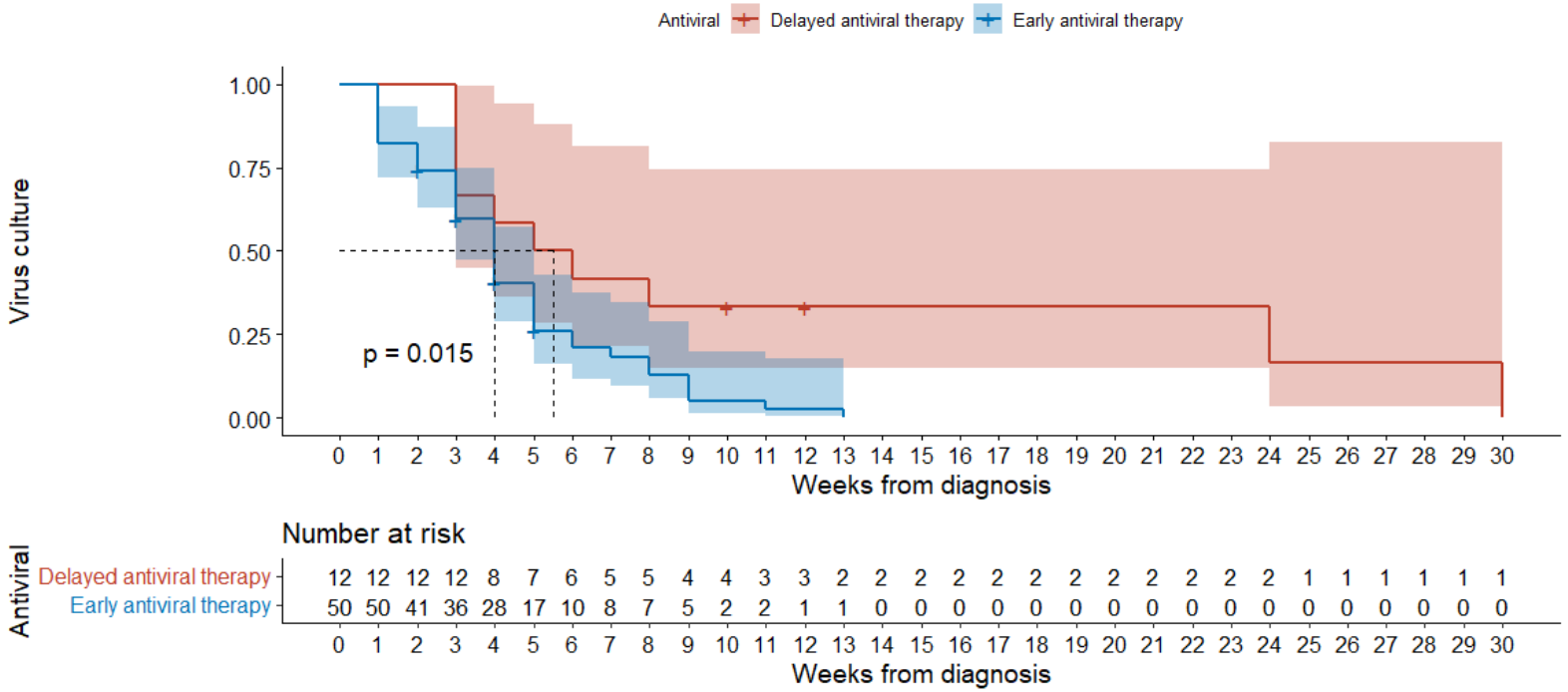
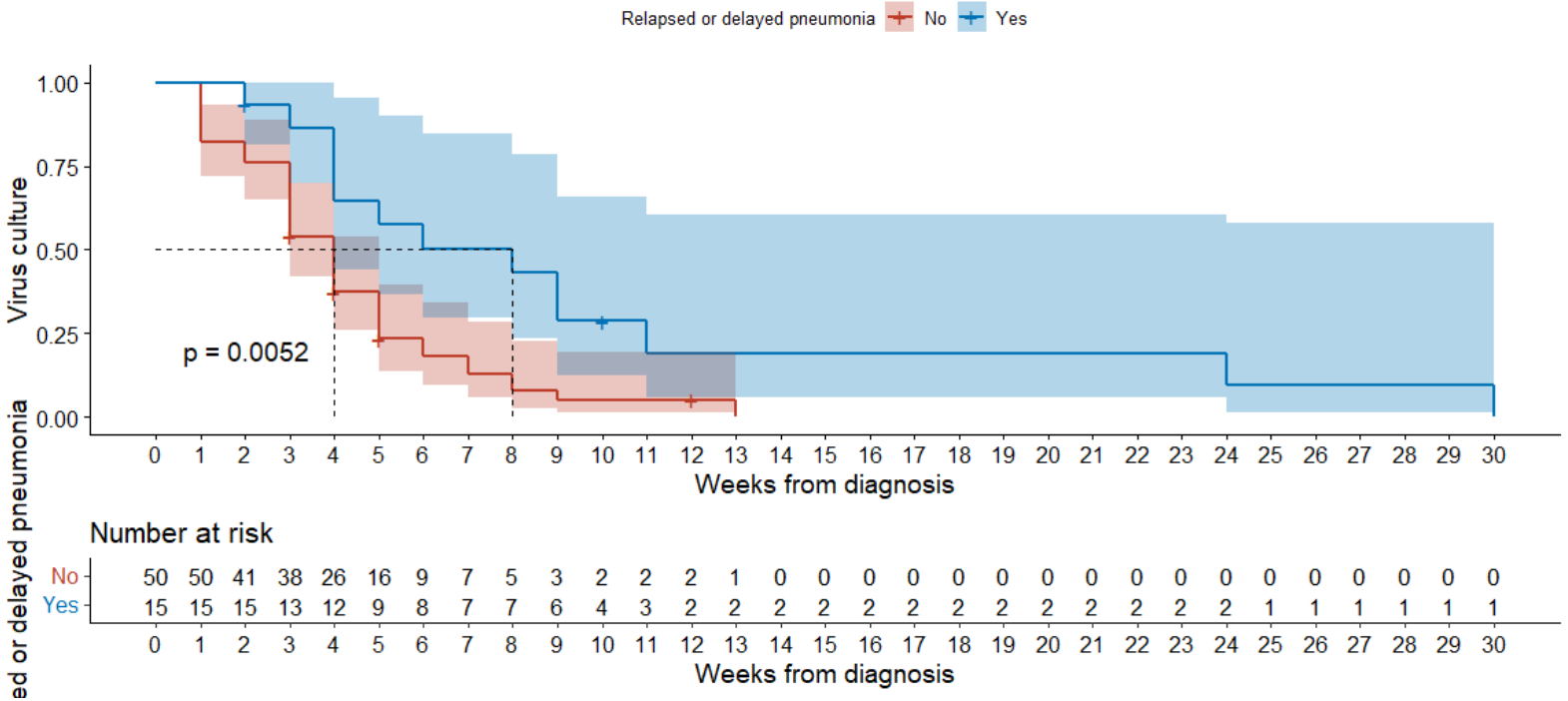

Antiviral
Delayed antiviral therapy
Early antiviral therapy
Virus culture
1.00
0.75
0.50
0.25
0.00
p = 0.015
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis
Number at risk
Antiviral
Delayed antiviral therapy 12 12 12 12 8 7 6 5 5 4 4 3 3 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1
Early antiviral therapy 50 50 41 36 28 17 10 8 7 5 2 2 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis

Figure 4D.


Relapsed or delayed pneumonia
No
Yes
Virus culture
1.00
0.75
0.50
0.25
0.00
p = 0.0052
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis
Number at risk
No 50 50 41 38 26 16 9 7 5 3 2 2 2 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Yes 15 15 15 13 12 9 8 7 7 6 4 3 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Weeks from diagnosis

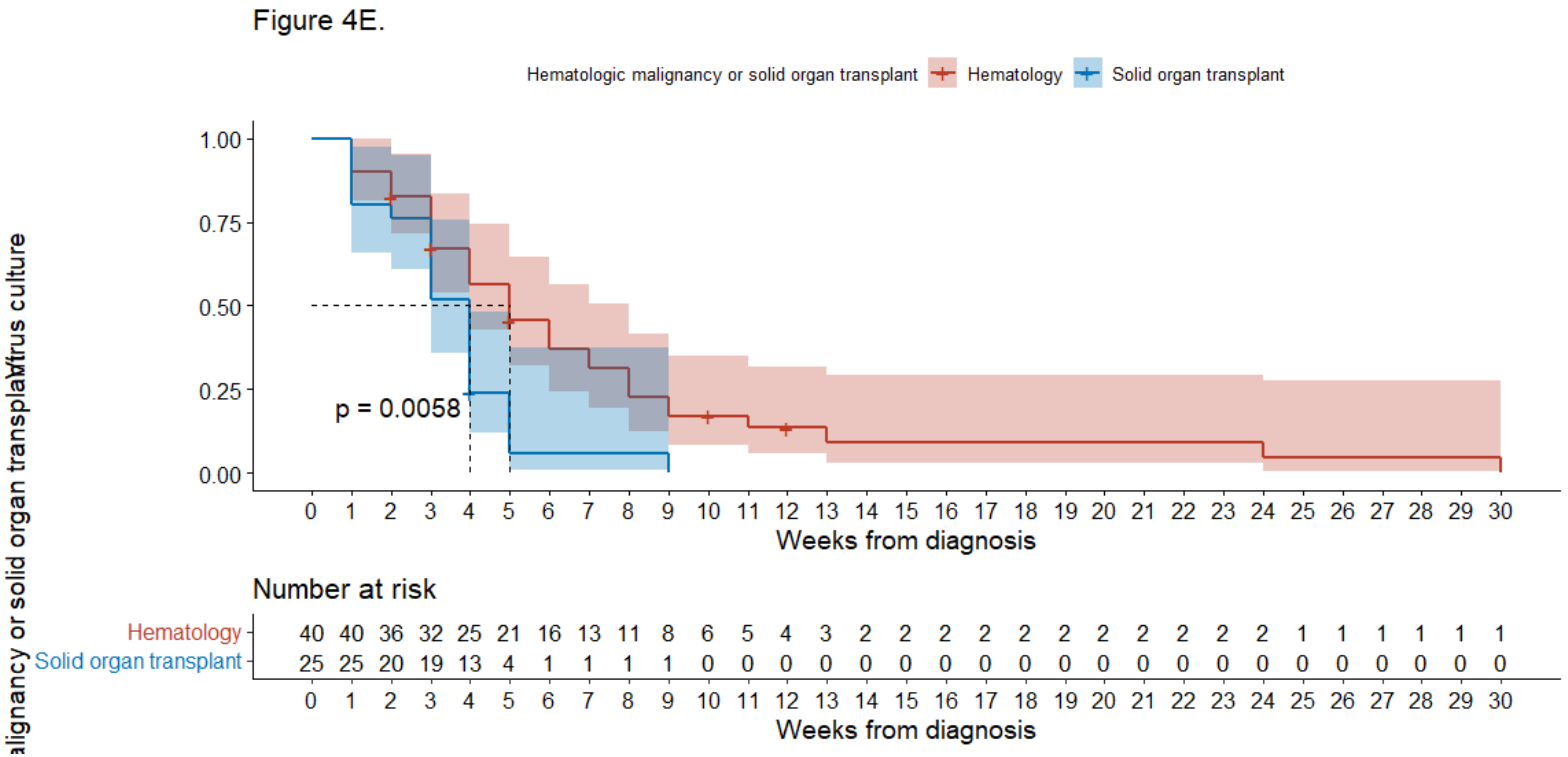


Figure 4. Comparison of viable virus shedding time in relation to various underlying conditions. 4A. Patients who received B-cell depletion therapy shed viable virus for longer (median 8 weeks IQR [5 – 9] vs. median 4 weeks IQR [3 – 5]). **4B.** Receiving T-cell depleting therapy did not affect the viable virus shedding period (median 4 weeks [IQR 3 – 6] vs. median 5 weeks [IQR 2 – 8]). **4C.** Early administration (within week of diagnosis) of antiviral agents, including remdesivir and ritonavir/nirmatrelvir, was associated with a shorter viable virus shedding period, compared with late administration (median 4 weeks, IQR [2 – 6] vs. median 5.5 weeks, IQR [3 – 24]). **4D.** Patients with relapsed or delayed pneumonia were more likely to display prolonged viral shedding (median 8 weeks, IQR [4 – 11], vs. median 4 weeks, IQR [3 – 5]). **4E.** Comparison of underlying immunodeficiency conditions. Patients with hematologic malignancy had longer viable viral shedding periods than those with solid organ transplants (median 5 weeks, IQR [3 – 8] vs. median 4 weeks, IQR [3 – 4]).

Figure 5A.

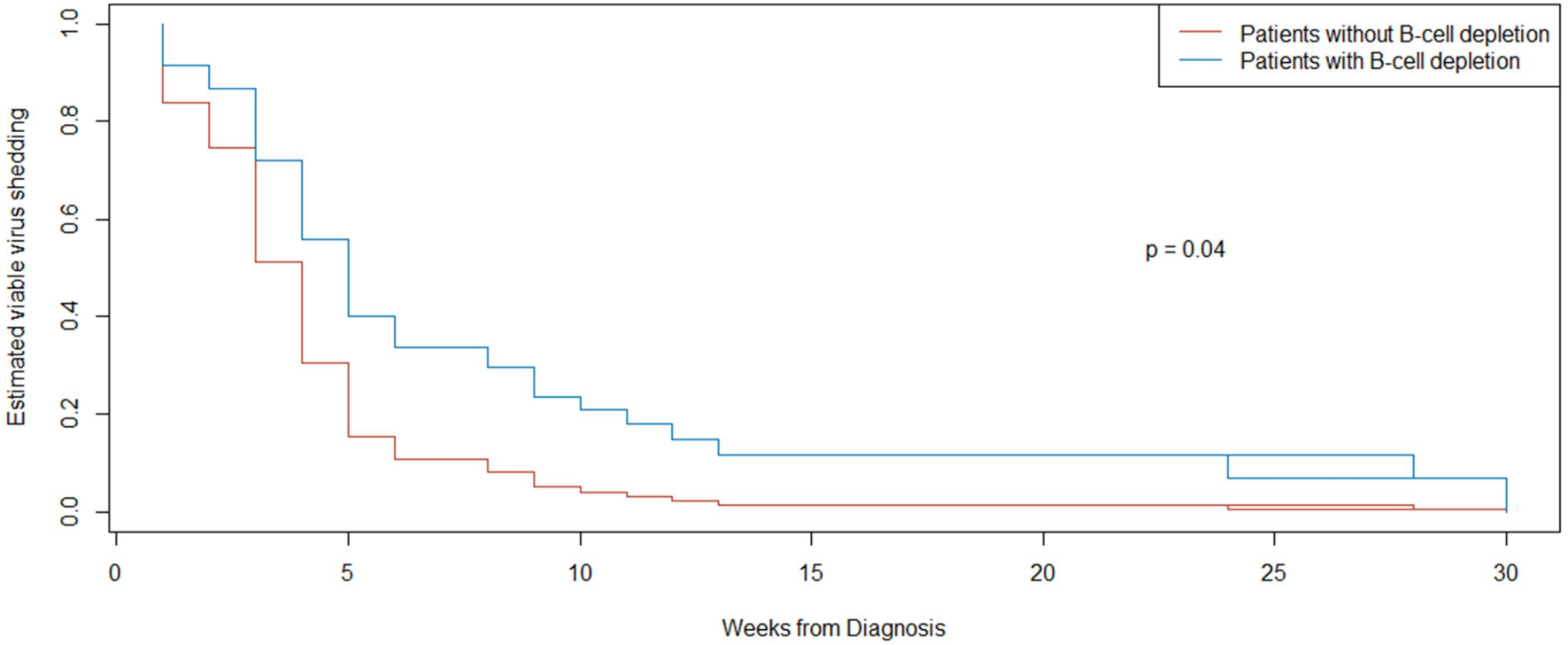

Patients without B-cell depletion
Patients with B-cell depletion
p = 0.04
Estimated viable virus shedding
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Weeks from Diagnosis

Figure 5B.

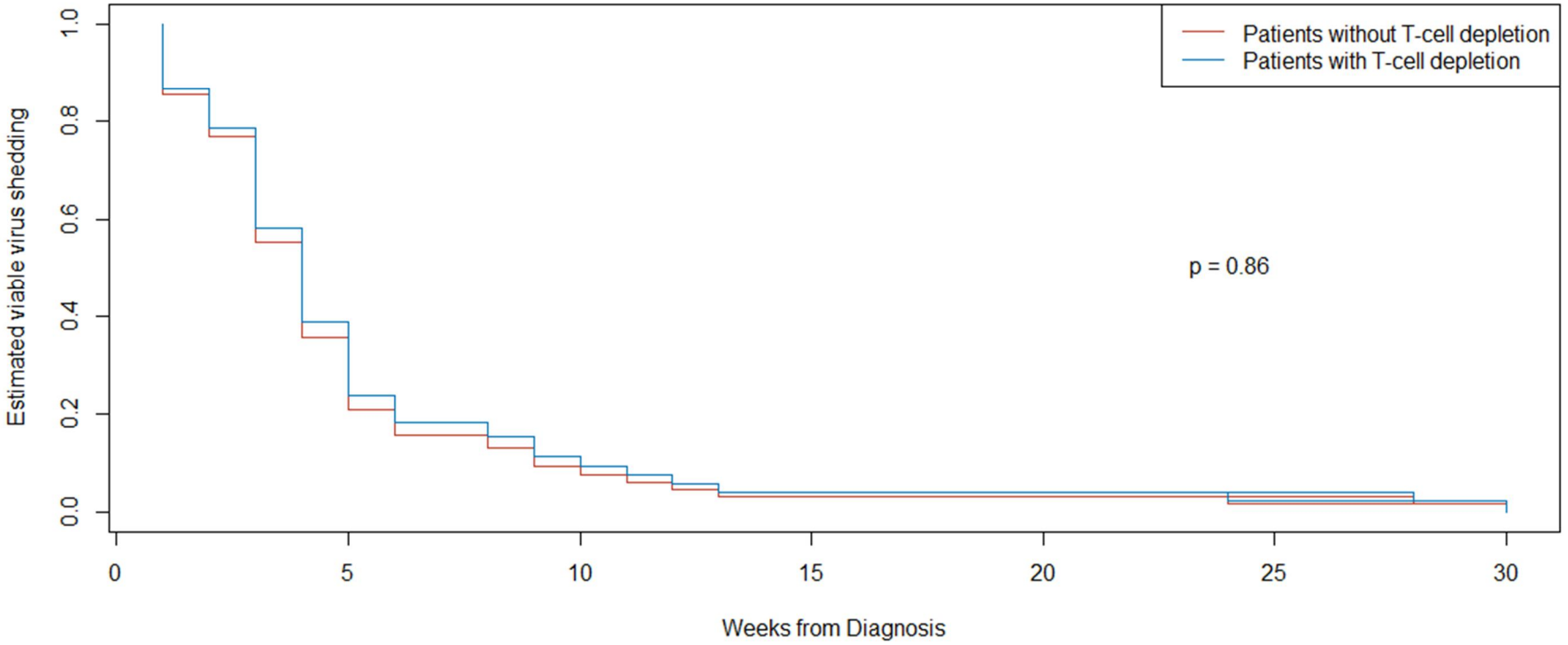

Patients without T-cell depletion
Patients with T-cell depletion
p = 0.86
Estimated viable virus shedding
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Weeks from Diagnosis

Figure 5C.

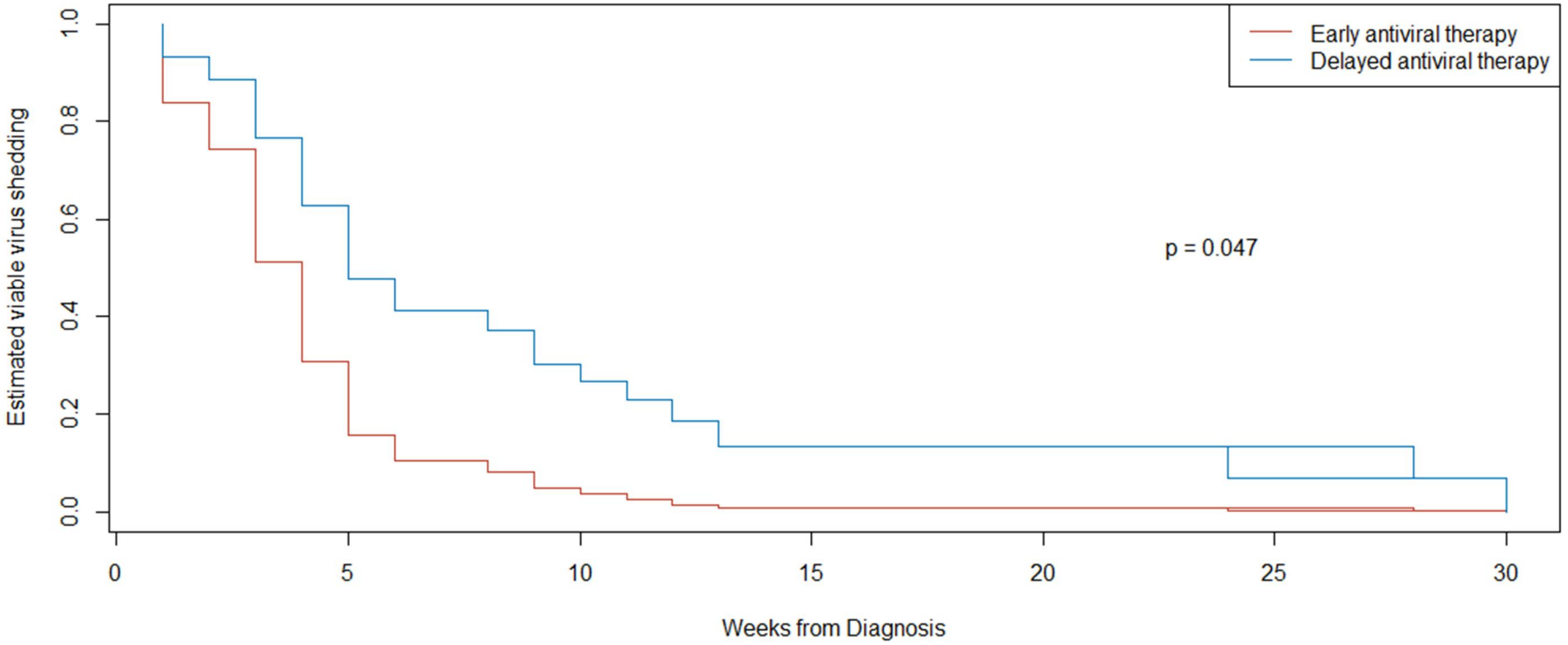

Early antiviral therapy
Delayed antiviral therapy
p = 0.047
Estimated viable virus shedding
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Weeks from Diagnosis

Figure 5D.

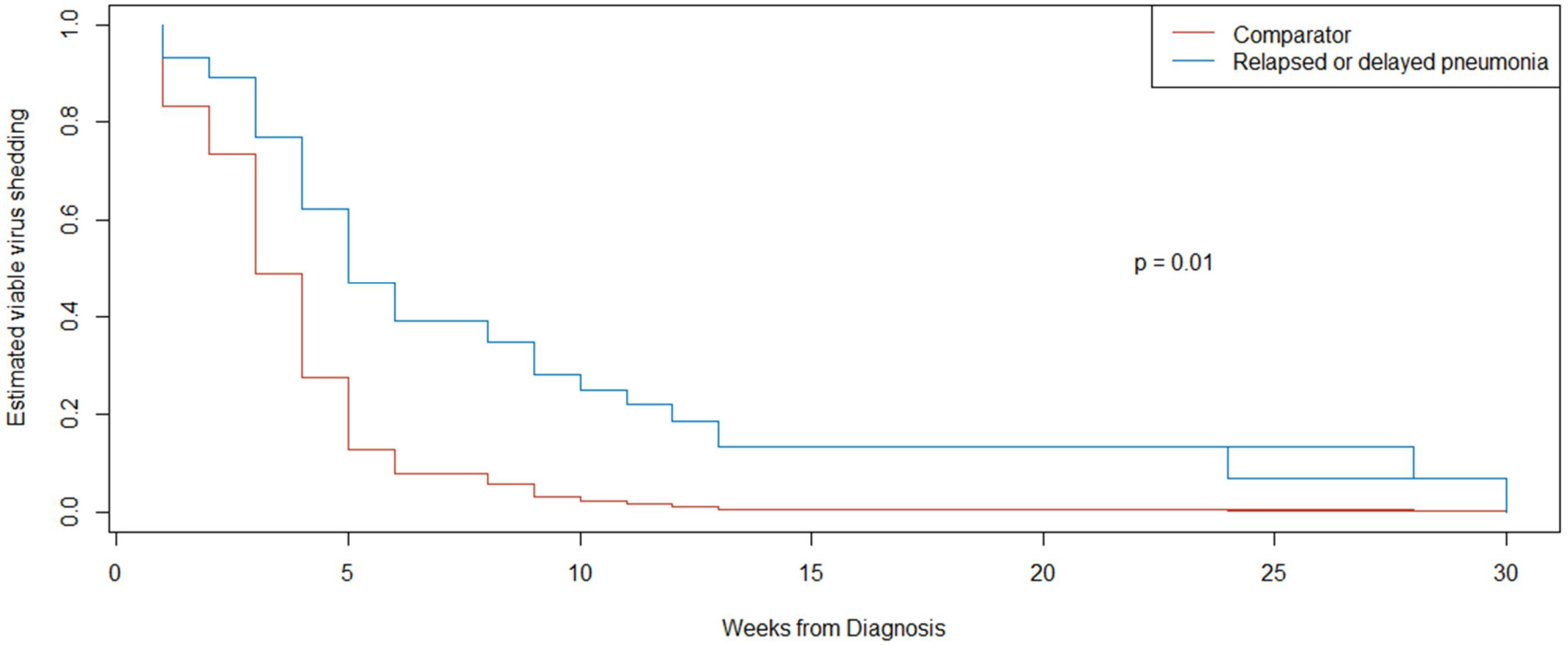

Comparator
Relapsed or delayed pneumonia
p = 0.01
Estimated viable virus shedding
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Weeks from Diagnosis

Figure 5E.

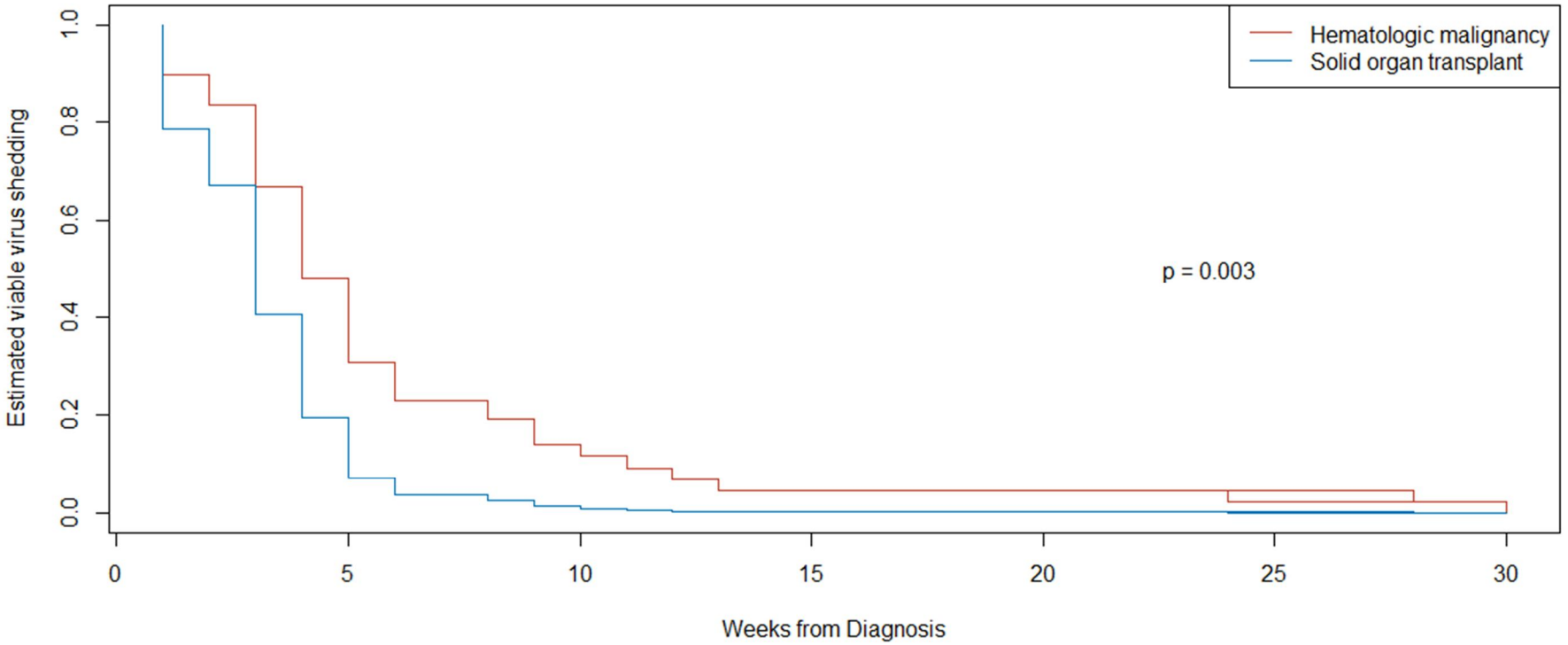


Figure 5. Semi-parametric model adjusted for interval censoring of virus shedding model according to given variables. 5A. B-cell depletion therapy (p = 0.04). 5B. T-cell depletion therapy (p = 0.86). 5C. Timing of antiviral therapy (p = 0.047). 5D. Relapsed or delayed pneumonia (p = 0.01). 5E. Comparison of underlying immunodeficiency conditions (p = 0.003).

A Cox proportional hazard model was constructed incorporating factors that were statistically significant in the univariate analysis (Table 3). COVID-19-specific therapy and B-cell depleting agent usage after COVID-19 diagnosis were defined as administration of the relevant agent prior to negative conversion of virus culture. The Cox model showed that use of B-cell depleting agent (aHR 4.76, 95% CI [1.11 – 20.00], p = 0.04) was associated with prolonged viral shedding, while vaccination (3 doses or more) was protective against prolonged viral shedding (aHR 0.22, 95% CI [0.06 – 0.77], p = 0.02).

Table 3. Univariate and multivariate analysis of viable virus shedding in immunocompromised COVID-19 patients

Characteristic	N	Univariate analysis			Multivariable analysis		
		Hazard Ratio	95% Confidence Interval	P-value	Adjusted Hazard Ratio	95% Confidence Interval	P-value
Age	65	1.01	0.98 – 1.03	0.56			
Male Sex	45	0.94	0.55 – 1.64	0.85			
Hematologic malignancy		2.30	1.29 – 4.09	0.005			
Initial severity							
Asymptomatic to mild	50	Reference					
Moderate to critical	15	1.35	0.71 – 2.56	0.35			
Vaccination status							
Less than 3-dose	41	Reference	-				
3-dose or more	24	0.63	0.37 – 1.09	0.098	0.22	0.06 – 0.77	0.02
B-cell depleting therapy							

Comparator	51	Reference					
B-cell depletion	14	2.56	1.28 – 5.26	0.008	4.76	1.11 – 20.00	0.04
Antiviral administration							
Late administration	12	Reference					
Early administration	50	0.41	0.19 – 0.88	0.03			
Dexamethasone	20	1.27	0.65 – 2.44	0.49			
Tocilizumab	8	3.33	1.00 – 11.11	0.05			
Baricitinib	11	1.69	0.76 – 3.84	0.19			

3. Clinical outcome of prolonged viral shedder

To evaluate clinical outcome of prolonged viral shedder, landmark survival analysis of all-cause mortality with different viral shedding period were examined. Log-rank test showed that viral shedding more than 6 weeks was significantly associated with increased all-cause mortality ($p = 0.04$: Figure 6). However, viral shedding duration shorter than 6 weeks was not significantly associated with an increased risk of all-cause mortality.

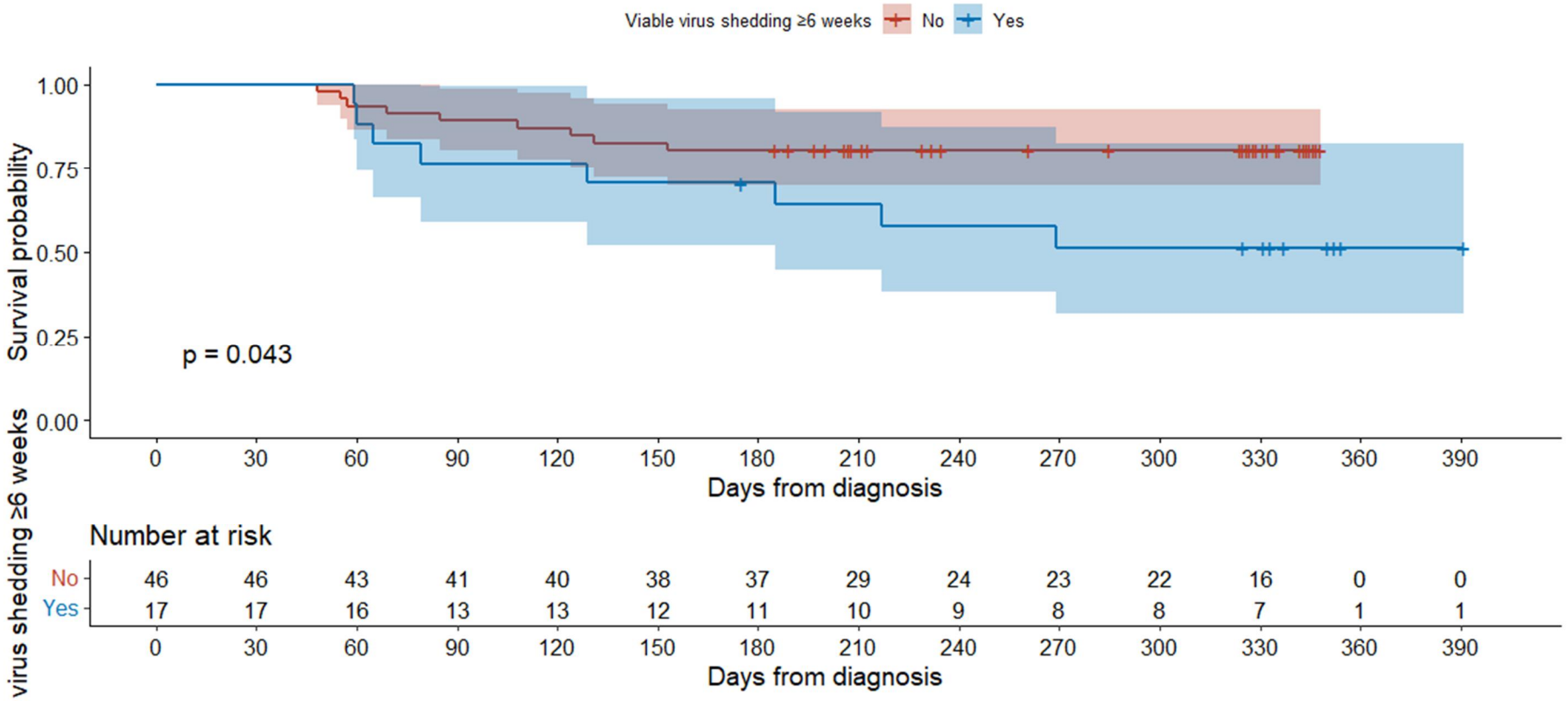


Figure 6. All-cause mortality according to viral shedding period. Patients with viable viral shedding more than 6 weeks were associated with higher all-cause mortality, while viral shedding less than 6 weeks was not associated with increased risk of mortality.

4. Diagnostic performance of the rapid antigen test

Rapid antigen tests were conducted on 262 of the 279 positive saliva samples in 61 patients (17 samples were excluded due to insufficient sample volume after RT-PCR) (Table 4). Overall, the rapid antigen test showed 79% (95% CI [71 – 86]) sensitivity and 76% (95% CI [69 – 82]) specificity, while positive predictive value was 74% (95% CI [66 – 81]) and negative predictive value was 81% (95% CI [73 – 87]). For the samples collected more than 20 days after diagnosis, sensitivity (81%, 95% CI [68 – 90], p = 0.83) and specificity (78%, 95% CI [70 – 85], p = 0.77) were insignificantly differ from the corresponding results obtained with samples obtained before day 20. However, positive predictive value (59%, 95% CI [46 – 70], p = 0.04) and negative predictive value (92%, 95% CI [85 – 96], p = 0.03) of samples after day 20 showed statistically significant differences, compared with samples obtained before day 20 (88%, 95% CI [78 – 94] and 48%, 95% CI [33 – 65] for PPV and NPV, respectively)

Table 4. Clinical performance of the rapid antigen test compared with virus culture

Sensitivity (overall)		**Specificity (overall)**	
TP/(TP+FN)	**% (95% CI)**	**TN/(TN+FP)**	**% (95% CI)**
96/121	79 (71 – 86)	105/138	76 (68 – 82)
PPV (overall)		**NPV (overall)**	
TP/(TP+FP)	**% (95% CI)**	**TN/(TN+FN)**	**% (95% CI)**
96/129	74 (66 – 81)	105/130	81 (73 – 87)
Sensitivity (≤ 20 days)		**Specificity (≤ 20 days)**	
TP/(TP+FN)	**% (95% CI)**	**TN/(TN+FP)**	**% (95% CI)**
60/77	78 (67 – 86)	16/24	67 (47 – 82)
PPV (≤ 20 days)		**NPV (≤ 20 days)**	
TP/(TP+FP)	**% (95% CI)**	**TN/(TN+FN)**	**% (95% CI)**
60/68	88 (78 – 94)	16/33	48 (33 – 65)
Sensitivity (> 20 days)		**Specificity (> 20 days)**	
TP/(TP+FN)	**% (95% CI)**	**TN/(TN+FP)**	**% (95% CI)**
36/44	81 (68 – 90)	89/114	78 (70 – 85)
PPV (> 20 days)		**NPV (> 20 days)**	
TP/(TP+FP)	**% (95% CI)**	**TN/(TN+FN)**	**% (95% CI)**
36/61	59 (46 – 70)	89/97	92 (85 – 96)

TP = true positive. FN = false negative. TN = true negative. FP = false positive.
PPV = positive predictive value. NPV = negative predictive value

Discussion

In this prospective cohort study, we found that immunocompromised patients with COVID-19 shed viable virus for a median 4 weeks. Risk factors from my previous study [11], including B-cell depletion and vaccination status, were associated with prolonged viral shedding. In addition, we demonstrated that the rapid antigen test after day 20 yielded a reliable negative predictive value. These data provide important information and insight into prolonged viable virus shedding in these immunocompromised patients, and into the potential use of RAT as a de-isolation strategy.

At the start of the COVID-19 pandemic, studies demonstrated that immunocompromised patients with COVID-19 had higher mortality and longer genomic RNA shedding periods [27]. However, shedding of genomic RNA is not a good surrogate marker of infectivity, although higher RNA viral copy number may correlate with higher virus burden [28]. The gold standard for infectivity of patients with COVID-19 is virus culture, which is not readily applicable in clinical practice due to its technical requirements [28]. Therefore, it is important to have information about the viable virus shedding period of immunocompromised patients with COVID-19, and to identify reliable and rapid test methods as surrogates for the transmissibility of those patients.

Jeong et al. demonstrated that less than 5% risk of premature ending of isolation is not achievable from single test strategy [20]. In the present study, we found that RAT performed before day 20 was associated with a relatively high positive predictive value but a low negative predictive value, whereas when performed after day 20 it had an acceptable negative predictive value. These results support the current CDC guidelines that recommend at least 20 days of isolation, and termination of isolation based on serial tests such as RAT. The

present study elucidated that the positive predictive value and negative predictive value of rapid antigen test vary depending on period of sample collection. The reason for these differences is because they reflect the prevalence of disease in the reference group in terms of positive predictive value and negative predictive value [29].

Prolonged viral shedding can lead to a variety of clinical problems. One concerns infection control, as it is important to note that patients with immunocompromised status are at high risk of severe COVID-19 and can transmit the SARS-CoV-2 to other patients in the same ward. Second, prolonged viral shedding may lead to intra-host evolution of SARS-CoV-2, which may result in the emergence of new variants. Finally, prolonged shedding can manifest as recurrent and persistent pneumonia. In this study, I examined atypical manifestations of COVID-19 pneumonia; protracted-course, relapsed-course, and delayed-onset, and found that viable virus shedding was significantly prolonged in patients with delayed or relapsed pneumonia. This suggests that the frequently-observed recurrent and persistent pneumonia in immunocompromised COVID-19 patients is caused by persistent active viral replication. There are several recent reports of resolution of long-term or combined antiviral therapy for COVID-19 pneumonia in immunocompromised patients [30-32]. Further studies are needed in this area.

In general, moderate-to-critical COVID-19 pneumonia has been thought to be mainly due to immune dysregulation rather than active viral replication [7]. However, most of the relevant data were obtained from elderly patients with normal immunity and pre-omicron strains of SARS-CoV-2. The results of the present study suggest that antiviral therapy against SARS-CoV-2 may help the resolution of immunocompromised patients with protracted or relapsed course of COVID-19 (Figure 4D). It is worth noting that RAT conducted prior to day 20 had

a fairly acceptable positive predictive value, indicating that it might be useful in the early stages of the disease in guiding the duration of antiviral treatment, even in immunocompromised patients whose symptoms last more than 5 or 7 days. Additional research is needed to establish treatment strategies that benefit these immunocompromised patients. Finally, the present study found that all-cause mortality is higher in patients with a long-term (more than 6 weeks) viable viral shedding period. Although there is high heterogeneity between two groups, this high mortality may be due not only to the deterioration of COVID-19 itself but also to problems that occur when appropriate treatment of the underlying disease is delayed during isolation. As mentioned earlier, this result emphasizes the need for research on therapeutics to reduce the active viral shedding period of COVID-19 in these patients.

This study has several limitations. First, this present study only included patients with active hematologic malignancies or solid organ transplants, not patients with advanced HIV infection or receiving immunosuppressant for other medical conditions. Second, although I excluded patients with a known history of prior COVID-19, the possibility of prior asymptomatic infection was not excluded and may act as a confounding factor. However, since this study was conducted during the early Omicron outbreaks in South Korea, the cumulative prevalence of COVID-19 in South Korea was below 1% [33]. Therefore, this factor may not have substantially affected main findings. Third, since I did not perform whole genome sequencing on the subsequent sample, it is difficult to rule out the possibility of reinfection in the prolonged viral shedders. Finally, we mainly collected saliva samples rather than NP swabs because the latter are logistically challenging in longitudinal sampling. As there is a lower chance of successful virus isolation from saliva sample [34], the present study may have underestimated the probability of viable virus shedding.

In conclusion, about half of COVID-19 patients with hematologic malignancy or transplants shed viable virus for more than 4 weeks, and viral shedding was prolonged especially in elderly unvaccinated patients who received B-cell depleted therapy. After more than 20 days from symptom onset, RAT had had a relatively high negative predictive value for viable virus shedding in immunocompromised patients.

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국문 요약

면역저하 COVID-19 감염환자의 바이러스 배출 동역학 및 격리 해제를 위한 신속항원 검사의 유용성

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목적: 중등도 이상의 면역저하 COVID-19 감염자는 장기간 생존 가능한 바이러스를 배출하는 것으로 알려져 있다. 이 환자군의 격리 기간에 대한 현행 미국질병관리청 (Centers for diseases control, CDC) 지침은 최소 20일의 격리 후 두 번의 연속된 검사 이후 감염내과 전문의의 협진을 통하여 격리 해제를 결정하는 것을 권유하고 있다. 그러나 이를 뒷받침하는 근거는 아직 부족한 상황이다.

방법: 고형 장기이식을 받았거나 혈액암에 대해 항암화학요법을 받고 있는 성인 COVID-19 환자를 대상으로 하였다. 모집된 환자들은 1주일 간격으로 타액을 뱉도록 안내되었으며, 기본 신체 정보 및 임상정보가 수집되었다. 수집된 타액에 대

해서 리보핵산(RNA) 실시간 중합효소연쇄반응 (RT-PCR) 을 통해 genomic 및 subgenomic RNA viral copy number 를 측정하였고, 이 가운데 genomic RNA PCR 결과가 양성인 환자들을 대상으로 바이러스 배양 검사 및 신속항원검사 (Rapid Antigen Test, RAT) 가 시행되었다. 배양 검사와 신속항원검사의 일치도에 대한 평가가 시행되었다. 생존 가능한 바이러스 배출 기간과 환자의 임상상과의 연관성을 파악하기 위해 폐렴의 양상을 지연성 (delayed), 재발상 (relapsed), 장기형 (protracted) 의 세 가지 형태로 분류하였고, 각각의 형태와 바이러스 배출 기간과의 연관성을 평가하였다. 마지막으로, 장기간의 바이러스 배출이 환자의 예후에 미치는 영향이 평가되었다.

결과: 총 65 명의 환자가 포함되었다. 이 가운데 40명의 환자는 혈액암에 대해 항암화학요법을 받았고, 나머지 25명의 환자는 장기 이식을 받은 환자였다. 바이러스 배출의 중위 기간은 4주였다 (사분위간 범위 [Interquantile range, IQR] 3 - 7). 다변수분석은 B-세포 결핍 (위험비율 [Hazard ratio, HR] 4.76, 95% 신뢰구간 [Confidence Interval, CI] 1.11 - 20.00) 이 생존가능한 바이러스 배출 기간을 유의미하게 연장시키는 반면, COVID-19 에 대한 3회 이상의 백신 접종은 바이러스 배출 기간을 유의미하게 감소시키는 것으로 나타났다 (HR 0.22. 95% CI 0.06 - 0.77). RAT의 민감도, 특이도, 양성 예측율, 음성 예측율은 각각 79%, 76%, 74%, 81% 였다. 20일 이전에 채취된 검체에서 RAT의 음성 예측율은 48% (95% CI 33-65) 이었으나, 21일 이후에 채취된 검체에 대해서는 92% (95% CI 85 - 96) 의 음성 예측율을 보였다. 한편, 재발성 혹은 지연성 폐렴은 유의미하게 바이러스 배출 기간을 연장시키는 반면 (중위 기간 8주 대 중위 기간 4주), 장기형의

폐렴을 보이는 환자는 바이러스 배출 기간이 유의미하게 길지 않은 것으로 나타났다. 생존 분석 결과 환자의 사망률 (all-cause mortality) 은 바이러스 배출 기간이 6주 이상인 그룹에서 유의미하게 높았으며, 그 이하의 기간에서는 유의미한 차이를 보이지 않았다.

결론: 절반 가량의 중등도 이상의 면역저하 COVID-19 감염 환자는 진단으로부터 4주 이상 생존 가능한 바이러스를 배출하는 것으로 나타났다. 특히 바이러스 배출 기간은 백신 접종을 완료하지 못한, B-세포 결핍 치료를 받은 환자에서 유의미하게 연장된 것으로 나타났다. 21일 이후에 채취된 검체에 대해 RAT는 배양 음성을 예측하는 유용한 도구가 될 수 있으며, 현행 CDC 지침대로 격리 해제의 근거로 사용될 수 있다.