Detection of significant antiviral drug effects on COVID-19 using viral load and PCR-positive rate in randomized controlled trials

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Abstract. To evaluate the effects of antiviral drugs against coronavirus disease (COVID-19), we calculated the sample size needed to detect significant differences in daily viral load in randomized controlled trials. While calculating sample sizes, simulated viral loads that mimicked longitudinal clinical data were used. The sample size computed from the viral load was the smallest 2 to 4 days after trial participation, while the smallest sample size computed from the positive rate was 4 to 7 days after clinical trial participation.

Key words: severe acute respiratory syndrome coronavirus 2, virus dynamics, clinical trial

Highlight

Inclusion criteria and early treatment are crucial in reducing the sample size required in the clinical trials in which daily viral load and positive rates are used as outcomes.

Introduction

Currently, many countries are severely affected by the coronavirus disease (COVID-19) pandemic. There is an urgent need to develop therapeutic drugs against COVID-19 because of the number of breakthrough infections and reinfections observed among vaccinated individuals and infected individuals, respectively [1, 2]. Antiviral drugs that reduce the viral load and shorten the duration of viral shedding are being developed to prevent further transmission. The development of these antiviral drugs is difficult because few clinical trials have evaluated antiviral drugs with consistent results [3].

We previously developed a quantitative simulation to evaluate the efficacy of antiviral drugs in randomized clinical trials and revealed that randomization and early initiation of treatment are the keys to obtaining significant differences in the duration of virus shedding and cumulative viral load as outcomes with reasonable sample sizes [4].

Using this simulator, we further discussed what is necessary to detect significant differences in daily viral load because the outcomes such as the duration of viral shedding and the cumulative viral load require longitudinal viral load data and therefore, huge efforts from healthcare workers. In several clinical trials, outcomes such as single-time points of viral load were compared as endpoints to evaluate the efficacy of antiviral drugs [5–8]. Assuming that antiviral drugs are sufficiently potent, we aimed to calculate the number of sample sizes required to detect the efficiency of antiviral drugs.

Materials and Methods

A mathematical model for virus dynamics with and without antiviral treatment

We employed a mathematical model previously used in [4] to calculate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load in COVID-19 patients with antiviral treatment blocking virus replication:

\[
\frac{df(t)}{dt} = -\beta f(t)V(t) \quad (1)
\]

\[
\frac{dv(t)}{dt} = (1 - e^{-\lambda H(t)}) \gamma f(t)v(t) - \delta v(t) \quad (2)
\]

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Here, \( r(t) \) represents the relative proportion of uninfected target cells compared from time \( t \) to time 0, corresponding to the time at symptom onset. The variable \( V(t) \) represents the viral load at time \( t \). The parameters \( \beta, \gamma, \) and \( \delta \) denote the rate constant for virus infection, the maximum rate constant for viral replication, and the decay rate of viruses, which is equal to the death rate of infected cells, respectively. Heaviside function, written as \( H(t) \) and defined as \( H(t) = 0 \) if \( t < t^* \); otherwise, \( H(t) = 1 \), shows the off and on treatment of the patients. \( t^* \) corresponds to the initiation of antiviral therapy, and \( \epsilon \) (0 ≤ \( \epsilon \) ≤ 1) is the inhibition rate of viral replication. We assumed \( \epsilon \) is close to 1, indicating that the antiviral drug is potent.

**Daily viral load of patients in simulation mimicking a randomized controlled trial for antiviral drugs**

The individual parameter distributions of model parameters were previously estimated from the viral loads of COVID-19 patients [9–12] using a non-linear mixed effect model [4]. A total of 10,000 parameter sets of \( V(0), \beta, \gamma, \) and \( \delta \) were randomly sampled from the estimated distributions, and the simulated longitudinal viral loads were generated using Eqs. (1),(2) as carried out in [4]. In this simulation, two different values of \( \epsilon \) (antiviral effect of drugs), 0.99 and 0.95, were used for sensitivity analysis. The time of trial participation was chosen from the estimated distribution of the time from symptom onset to hospitalization [13]. Here, we assumed that treatment was immediately initiated at the time of trial participation. Truncated distributions were also used to mimic trials with different inclusion criteria within 0.5, 1, 2, 3, and 4 days after symptom onset.

**Calculation of sample sizes from daily viral load**

The daily PCR-positive rates after participation in trials were calculated using the simulated longitudinal viral load and used to detect statistically significant differences between the groups with and without treatment. Here, we assumed the detection limit of viral load in PCR tests to be 100 copies/ml. Sample sizes were derived by two-sided Welch’s \( t \)-test with a significance level of 0.05, and a power of 80%, which were calculated using the \( AB_{12n}\_prop( ) \) function in the R package pwrAB. Additionally, we derived the sample sizes from the means and standard deviations of \( \log_{10}\)-daily viral load, which were calculated using the \( AB_{12n}() \) function in the R package pwrAB. The values of viral load under the detection limit were assumed to be 1 copy/ml for the calculation of averages and standard deviations.

**Results**

The difference in the timing of the significant antiviral efficacy detection for the smallest sample size between the PCR-positive rate and \( \log_{10}\)-daily viral load

We calculated and compared the sample sizes for detecting the significant effect of antiviral drugs based on the PCR-positive rate and simulated \( \log_{10}\)-daily viral load. The sample sizes calculated for the PCR-positive rate were the smallest at 4–7 days after trial participation (Fig. 1A, 1B). In contrast, the sample size calculated for the \( \log_{10}\)-daily viral load was the smallest at 2–4 days (Fig. 1C, 1D). Immediately after the trial participation, the PCR-positive rates remained near 100% regardless of the groups with and without treatment, and there was almost no difference between these two groups (Supplementary Fig. 1A, 1B). Then, the PCR-positive rates began to decrease earlier in the treatment groups than in the groups without treatment. The differences were largest around 4 to 7 days, which resulted in the smallest sample size (Fig. 1A). For \( \log_{10}\)-daily viral load, while the difference was not maximized at the beginning of the trials, the smaller standard deviations led to smaller sample sizes (Supplementary Fig. 1C, 1D). As the trials progress, the variance increased and the sample size became larger day by day.

**Comparison of sample sizes in different trials**

The required sample size varied depending on the inclusion criteria (Fig. 1). The sample sizes were approximately 500 or less and 400 or less when the inclusion criteria were employed (0.5 days to 4 days) and more than 2,000 and 1,000 without the inclusion criteria (all patients), for daily PCR-positive rate and \( \log_{10}\)-daily viral load, respectively (Supplementary Tables 1 and 2).

The required sample size was larger in the later inclusion criteria. Moreover, the sample size computed for both PCR-positive rate and \( \log_{10}\)-daily viral load was larger in the trials with the antiviral drug with a 95% inhibition rate than with the drugs with a 99% inhibition rate (Fig. 1). However, the difference in sample size itself was subtle. In general, the sample sizes calculated for the PCR-positive rate were larger than those calculated using the \( \log_{10}\)-daily viral load.

**Conclusion**

For both PCR-positive rate and \( \log_{10}\)-daily viral load, the calculated sample sizes varied depending on the inclusion criteria. Moreover, the days with the smallest sample size were different. When the sample size was calculated for PCR-positive rate, the smallest value was 7 days using the strictest inclusion criterion (within 0.5 days after trial participation). In contrast, when it was calculated for the \( \log_{10}\)-daily viral load, the smallest value was 2 days. This difference in the sample size was due to the differences in the properties of the PCR-positive and \( \log_{10}\)-daily viral loads. A clinical trial based on the daily viral load should be planned, considering the dynamic properties of virus infection if we used the trial outcomes to evaluate the antiviral effects on patients’ viral load.

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**Fig. 1.** The time-change of sample size calculated using the PCR-positive rate and the log_{10}-daily viral load. The sample sizes at each time in the randomized controlled trials for the antiviral drugs (99% (A and C) or 95% (B and D) replication inhibition) with different inclusion criteria (within 0.5, 1, 2, 3, 4 days after trial participation) were calculated from the simulated PCR-positive rate (A and B) and the log_{10}-daily viral load (C and D).

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Potential Conflicts of Interest

The authors have nothing to disclose.

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