**STROBE Statement—Checklist of items that should be included in reports of cohort studies**

| Item No | Recommendation | Page No |
|---------|----------------|---------|
| 1       | (a) Indicate the study’s design with a commonly used term in the title or the abstract  
‘Is viral load of SARS-CoV-2 a predictor of mortality in COVID-19 ARDS patients?’  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found  
**Objective.** The viral load varies during infection and is higher during the initial stages of the disease. Considering that the need for ICU comes in the late stages of COVID-19, detecting the viral load by analyzing Ct values at ICU admission can be a clinically valuable tool for identifying patients with the highest mortality risk.  
**Methods.** This is a retrospective, double-center, observational cohort study among COVID-19 patients admitted to the intensive care unit. Patients included in the study was older than 18 years old, had a positive SARS-CoV-2 PCR and PaO$_2$/FiO$_2$ ratio $< 200$. The patient population was divided into two groups: 1) survivors and 2) nonsurvivors.  
**Results.** Two hundred patients were included in the study. In nonsurvivors, age, APACHE II, SOFA score, CCI, and procalcitonin level were significantly higher whereas PaO$_2$/FiO$_2$ ratio, and Ct level was significantly lower than in survivors.  
**Conclusion.** When combined with age, comorbidities, and severity scores, viral load at ICU admission may have prognostic implications and may help clinicians identify patients who require more intensive monitoring. Increased awareness may improve outcomes by closely monitoring and treating patients more effectively. A more prospective study is needed to determine how a high viral load worsens the disease and how to avoid irreversible results. |

**Introduction**

| Item No | Recommendation | Page No |
|---------|----------------|---------|
| 2       | **Background/rationale**  
Explain the scientific background and rationale for the investigation being reported  
In some viral diseases, viral load monitoring can provide information about the life expectancy for a patient [1]. The standard-of-care test for detecting SARS-CoV2 includes semi-quantitative data on viral load such as cycle threshold (Ct) values and lower Ct values are associated with more severe diseases [2–5]. Although the SARS-CoV-2 viral load is being researched broadly, the majority of studies contain non-intensive care unit (ICU) patients [2, 3, 5–7]. Actually, the viral load varies during the course of infection and is higher during the initial stages of the disease [2, 3, 6]. On the other hand, the importance of viral load in the late stages of infection or persistent positive, as well as the clearance of SARS-CoV-2 from respiratory samples, is still unclear. |
| 3       | **Objectives**  
State specific objectives, including any prespecified hypotheses  
Considering that the need for ICU comes in the late stages of COVID-19, detecting the viral load by analyzing Ct values at ICU admission can be a clinically valuable tool for identifying patients at the highest risk of |
For this reason, in this study, we evaluated the relationship between Ct values and mortality in patients with Covid-19 in the ICU. We hypothesized that ICU admission SARS-CoV-2 viral load has a predictive performance to ICU mortality.

## Methods

### Study design

**Present key elements of study design early in the paper**

‘We performed retrospective, double-center, observational cohort study.’

### Setting

**Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection**

‘… study among COVID-19 patients admitted to the intensive care units (ICUs) of two hospitals between April 2020- June 2021. The study was approved by the Ministry of Health, the special COVID-19 Ethics Committee of Istanbul University, and the Ethics Committee of Acibadem University (approval number: 2021-09/36, date: 26.05.2021).’

### Participants

**Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up**

‘All of the patients who were older > than 18 years old, had a positive SARS-CoV-2 PCR and PaO2/FiO2 ratio < 200 were randomly included in the study whereas patients whose samples were negative for SARS-CoV-2 at ICU admission, whose samples were positive but analyzed at a different hospital, and whose PaO2/FiO2 ratio > 200 were excluded from the study.’

**For matched studies, give matching criteria and number of exposed and unexposed**

Not a matched study.

### Variables

**Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.**

Our results showed that viral load at ICU admission can predict the mortality of patients with ARDS due to COVID-19. It is hardly surprising that we found a high rate of mortality (56.5 percent). The incidence of ARDS among COVID-19 ranges between 40% and 96% [18] [19] because of differences in health systems’ organization, availability of ICU beds, and lengths of follow-up [20]. Therefore, it is important to note that the patients included in our study were more severe, with a PaO2/FiO2 ratio of 97 (86–125) (p<0.001). Previously, a low PaO2:FiO2 ratio at ICU admission was shown to be an independent factor associated with mortality [21]. We also demonstrated a significant difference (p<0.001; Table 2) between the Ct values of the two groups, survivors (n=87) and non-survivors (n=113), who were admitted to the ICU, according to the SARS-CoV-PCR test results. Apart from the viral load, the association between mortality and COVID-19 ARDS is driven by age (p < 0.001) and comorbidities (CCI < 0.001). This was the expected result because they have already been shown to be important risk factors for severe COVID-19 [24].
Although we showed that viral load is predictive of ICU mortality, no live virus was isolated from sample cultures obtained eight days after the onset of symptoms [30]. However, this result can be attributed to the fact that there were not enough viruses to reproduce in the viral culture. Nevertheless, the persistence of viral RNA may not be associated with disease severity but may indicate that the immune response is unable to promote the virus RNA clearance. The lack of information regarding the persistence of virus RNA and infectivity, disease severity, and immune response supports the current guidance for viral clearance confirmation prior to patient transference out of dedicated COVID-19 wards and for ending isolation for patients with mild illness [31].

### Data sources/ measurement

*For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.*

The data were collected from electronic and manual medical records. Patients’ demographics (age, gender, body mass index), ICU admission scores such as the Acute Physiology and Chronic Health Evaluation System (APACHE) II, the Sequential Organ Failure Assessment (SOFA), and the Charlson Comorbidity Index (CCI) score, \( \text{PaO}_2/\text{FiO}_2 \) ratio, leucocyte counts, lymphocyte counts, neutrophil-lymphocyte count ratio (NLCR), \( C_t \), C-reactive protein (CRP), procalcitonin, ferritin, d-dimer, and lactate dehydrogenase (LDH) levels at the ICU admission and outcomes such as length of ICU stay and mortality rate were recorded. For the purpose of this study we have divided the \( C_t \) values into 2 groups, as low (<20 cycles) and high (>20 cycles). The patient population was divided into two groups: 1) survivors and 2) nonsurvivors.

### Bias

*Describe any efforts to address potential sources of bias*

Bias can occur in the planning, data collection, analysis, and publication phases of research.

1. **Planning**: We clearly defined outcomes and hypothesis prior to study implementation.
2. **Data collection**: We collect all of the COVID-19 patients admitted to the intensive care units (ICUs) of two hospitals between April 2020-June 2021, who were older than 18 years old, had a positive SARS-CoV-2 PCR and \( \text{PaO}_2/\text{FiO}_2 \) ratio < 200 were randomly included in the study.
3. **Analysis**: SPSS, Version 23.0, was used to conduct statistical analyses (IBM Corp., Armonk, NY, USA).
4. **Publication phases of research**: The reporting of this study conforms to STROBE guidelines.

### Study size

*Explain how the study size was arrived at*

All of the COVID-19 patients admitted to the intensive care units (ICUs) of two hospitals between April 2020-June 2021, who were older...
> than 18 years old, had a positive SARS-CoV-2 PCR and PaO₂/FiO₂ ratio < 200 were randomly included in the study.

| Quantitative variables | 11 Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
|                        | For the purpose of this study we have divided the Ct values into 2 groups, as low (<20 cycles) and high (>20 cycles). The patient population was divided into two groups: 1) survivors and 2) nonsurvivors. According to the distribution of the values, data are presented as means, medians, and interquartile ranges (IQRs). The Kolmogorov-Smirnov test was employed to determine whether the distribution was normal. We employed student t, chi-square, and Mann-Whitney U tests for both groups' analyses. ROC curve analysis was used to find the cut-off and area under the curve values of all relevant variables in the non-survivors group. In a multivariate logistic regression model predicting the likelihood of mortality, we added all significantly different parameters in the non-survivors group. |

| Statistical methods | 12 (a) Describe all statistical methods, including those used to control for confounding |
|---------------------|--------------------------------------------------------------------------------------------|
|                     | SPSS, Version 23.0, was used to conduct statistical analyses (IBM Corp., Armonk, NY, USA). According to the distribution of the values, data are presented as means, medians, and interquartile ranges (IQRs). The Kolmogorov-Smirnov test was employed to determine whether the distribution was normal. We employed student t, chi-square, and Mann-Whitney U tests for both groups' analyses. ROC curve analysis was applied to significantly different parameters in the non-survivors group, and the cutoff values were detected by using Youden’s index. In a multivariate logistic regression model predicting the likelihood of mortality, we added all significantly different parameters in the non-survivors group. A p-value of less than 0.05 was used to determine statistical significance. The estimated power of this study was detected as 0.99 (as per groups’ sizes [87 and 113], mean difference for Ct values of groups [3.8] and α=0.05). |
|                     | (b) Describe any methods used to examine subgroups and interactions |
|                     | No subgroup analysis. |
|                     | (c) Explain how missing data were addressed |
|                     | The study has retrospective nature. Patients with missing data were excluded. |
|                     | (d) If applicable, explain how loss to follow-up was addressed |
|                     | The study has retrospective nature. |
|                     | (e) Describe any sensitivity analyses |

| Results | 13* Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
|---------|---------------------------------------------------------------------------------------------------------------------------------|
|         | Two hundred patients were included in the study (Figure 1). |
(b) Give reasons for non-participation at each stage
The study has retrospective nature.
(c) Consider use of a flow diagram

**Descriptive data** 14*

(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders

For all patients, mortality rate and Ct level were found as 56.5% (n=113) and 23.7±5.9 respectively (Table 1).

(b) Indicate number of participants with missing data for each variable of interest

Flow diagram, Figure 1

(c) Summarise follow-up time (eg, average and total amount)

Intensive care duration was the follow-up time.

**Outcome data** 15*

|  |  |
|---|---|
| **Report numbers of outcome events or summary measures over time** | 5 |
| In our study, a significant difference (p<0.001; Table 2) was found between the CT values of the two groups, survivor (n=87) and non-survivor (n=113), who were admitted to the ICU, according to the SARS-CoV-PCR test results. | |
| In non-survivors, age, APACHE II, SOFA score, CCI, and procalcitonin level were significantly higher whereas PaO$_2$/FiO$_2$ ratio, and Ct levels were significantly lower than in survivors (p<0.001, p=0.002, p=0.020, p<0.001, p<0.001, p<0.001 and p<0.001) (Table 2). | |
Main results

1. Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included.

For mortality, cut-off values of CCI, procalcitonin, PaO\textsubscript{2}/FiO\textsubscript{2} ratio, Ct level, age, APACHE II and SOFA score were ≥4, ≥28, <107, ≥24.7, ≥17 and ≥7 respectively (p<0.001, p<0.001, p<0.001, p<0.001, p=0.002 and p=0.021 respectively) (Table 3). In multivariate logistic regression model, likelihood of mortality was increased 4.2-fold (2.1-8.2), 3.7-fold (1.7-8.0), 2.4-fold (1.3-4.7) and 2.0-fold (1.1-3.9) by Ct≤24.7, CCI≥4, PaO\textsubscript{2}/FiO\textsubscript{2} ratio<107 and procalcitonin≥0.28 (p<0.001, p=0.002, p=0.008 and p=0.033 respectively) (Table 4).

Other analyses

1. Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses.

No other analyses.

Discussion

Key results

1. Summarise key results with reference to study objectives.

‘Our results showed that viral load at ICU admission can predict the mortality of patients with ARDS due to COVID-19.’

‘We also demonstrated a significant difference (p<0.001; Table 2) between the Ct values of the two groups, survivors (n=87) and non-survivors (n=113), who were admitted to the ICU, according to the SARS-CoV-PCR test results. A positive reaction in a real-time PCR assay is detected by the accumulation of a fluorescent signal. The Ct value represents the number of replication cycles required for sufficient gene amplification to produce a fluorescent signal that crosses a predefined threshold. This is a semi-quantitative measure of viral genetic material and it is inversely proportional to the amount of target nucleic acid in the sample. Therefore, it is possible to make a preliminary estimation of viral load, although it is not a complete quantitative measurement with the Ct value.’

Limitations

1. Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

This study had several limitations, which must be acknowledged. First, because the study has retrospective nature. Second, because the study only included a single sample of PCR testing, we can't see the viral dynamics during infection. Third, pre-ICU management is missing.

Interpretation

2. Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

‘A similar result was reported by Huang et al., who showed that lower Ct values were correlated with an increased risk of death [22]. In another study, viral load was not associated with mortality, but older age, CRP positivity, and CT severity were significant risk factors [23]; however, the patients included in this study were not pure ARDS patients.’

‘Apart from the viral load, the association between mortality and COVID-19 ARDS is driven by age (p < 0.001) and comorbidities (CCI < 0.001). This was the expected result because they have already been shown to be important risk factors for severe COVID-19 [24]. Similar findings have even been made for MERS-CoV, in which disease severity increases with age, and severe disease has been uncommon among pediatric patients [25]. Older patients and patients with comorbidities may have decreased physiologic reserves and are thus less likely to
tolerate the insults caused by COVID-19. Immunosenescence seen in these patients is associated with a decreased response to pathogens [26]. In addition, older people have higher levels of ACE2 in their alveoli [27], which are thought to be receptors for entry into host cells [10]. Zheng et al. found a correlation between age and the duration of the virus. The reasons for higher viral loads in severe individuals are not well understood and warrant further investigation.

‘Although we showed that viral load is predictive of ICU mortality, no live virus was isolated from sample cultures obtained eight days after the onset of symptoms [30]. However, this result can be attributed to the fact that there were not enough viruses to reproduce in the viral culture. Nevertheless, the persistence of viral RNA may not be associated with disease severity but may indicate that the immune response is unable to promote the virus RNA clearance. The lack of information regarding the persistence of virus RNA and infectivity, disease severity, and immune response supports the current guidance for viral clearance confirmation prior to patient transference out of dedicated COVID-19 wards and for ending isolation for patients with mild illness [31].’

| Generalisability | 2 | Discuss the generalisability (external validity) of the study results |
|------------------|---|---------------------------------------------------------------------|
|                  | 1 | In COVID-19 critically ill patient combination of age, comorbidities, and severity scores, viral load may assist clinicians in identifying individuals who need more intensive monitoring. Increased awareness may improve outcomes by closely monitoring and treating patients more effectively. |

**Other information**

| Funding | 2 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |
|---------|---|-------------------------------------------------------------------------------------------------------------------------------------|
|         | 2 | No fundings.                                                                                                                                 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.