

ORIGINAL ARTICLE

Comparison of Forced Expiratory Flow (FEF) 25-75% between Post-COVID-19 Patients with Different Severity at Universitas Gadjah Mada Academic Hospital

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ABSTRACT

Introduction: Shortness of breath is the most distressing long COVID-19 symptom associated with the decline of small airway function, as shown by a decrease in forced expiratory flow (FEF) 25-75% value in the spirometry test. This study aimed to compare FEF 25-75% values as a predictor of small airway disease between mild-moderate and severe-critical long COVID-19 patients.

Methods: This study used a prospective cohort design that included 24 post-hospitalized COVID-19 patients who came to the long COVID-19 clinic at Universitas Gadjah Mada Academic Hospital (UGM AH), Yogyakarta. The subjects were divided into mild-moderate and severe-critical groups based on the World Health Organization (WHO) classification. The subjects were tested for spirometry three months after the onset of COVID-19 symptoms. The comparison of both severity groups used the percent prediction of FEF 25-75% spirometry results. The value was interpreted as abnormal if the predicted FEF 25-75% value was below 65%.

Results: There were three (25%) and two (16.67%) subjects with FEF 25-75% predicted below normal values in the mild-moderate and severe-critical groups consecutively, which showed a decline in small airway function. This study showed no statistically significant differences (p -value = 0.882) between the means of FEF 25-75% predicted values of the two groups.

Conclusion: A small proportion of post-COVID-19 syndrome patients had small airway disease, and there were no statistical differences in small airway function between the groups.

INTRODUCTION

Coronavirus disease 2019, or COVID-19, is a respiratory disease responsible for the pandemic that has been occurring since 2020. According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), most COVID-19 infections resolve entirely.^{1,2} However, some may have physical or mental long-term effects that persist for weeks after the acute infection. In October 2021, WHO created a long COVID-19 definition involving clinicians, researchers, and COVID-19 patients. Long

COVID-19 is defined as symptoms experienced by confirmed or probable COVID-19 patients within three months after COVID-19 infection. These symptoms persist for at least two months, and another diagnosis cannot explain these symptoms. Symptoms can include weakness, shortness of breath, cognitive dysfunction, or other symptoms that interfere with daily activities. No minimal symptoms are required to make a long-term diagnosis of COVID-19. Symptoms may appear after recovering from acute COVID-19 infection or have persisted since acute COVID-19 and fluctuate occasionally.³

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A systematic review by Chen, *et al.* (2022) reported the prevalence of long COVID from 1,680,003 COVID-19-positive patients from 33 studies to be 43% and reported that the prevalence was higher in patients who were hospitalized (54%) rather than those who were not hospitalized (34%).⁴ Studies also reported breathlessness as one of the more common persistent symptoms of long COVID-19.⁵ Fernandez-de-las-Penas, *et al.* (2022) reported that 55% of patients complained of breathlessness during activity, and 23.5% of patients complained of breathlessness during rest.⁶ Aside from being one of the most reported symptoms of long COVID-19, breathlessness is also one of the symptoms that causes a significant decrease in patients' quality of life.⁷

Elicker (2022) described two patterns of histopathological manifestations in pneumonia, including COVID-19, such as diffuse alveolar destruction and concentric fibrosis around bronchioles causing the obstruction.⁸ These histopathological manifestations can be affected by the severity of acute COVID-19 infections because the pathophysiology of long COVID-19 is associated with the sequelae of organ damage and its extent, including persistent chronic inflammation and formation of autoantibodies, which are more common in severe or critical patients.⁹ Many studies have shown diffuse alveolar destruction due to COVID-19 infections and its manifestation as a restrictive pattern in spirometry tests. However, studies concerning obstructive patterns, specifically small airway disease due to COVID-19 infections, are minimal, including in Indonesia.

Small airway abnormalities can be reflected through the forced expiratory flow (FEF) 25-75% of patients' spirometry tests. The FEF 25-75% is one of the most common parameters used to evaluate any pathology on the small airways. However, this parameter also has its weaknesses, such as its low specificity toward small airway diseases and low sensitivity within the first stages of developing small airway disease or if the changes are mild. It is still commonly used due to its accessibility and ease of use.¹⁰ This study aimed to compare small airway function between mild-moderate and severe-critical patients in long COVID-19 patients by assessing FEF 25-75%.

METHODS

This was an observational study using a prospective cohort design and a consecutive sampling method. The subjects were recruited from post-hospitalized COVID-19 patients at Universitas Gadjah Mada Academic Hospital (UGM AH), Yogyakarta. This study was conducted from August 2021 to January 2022.

It is noteworthy that, during this time, both in Indonesia and the world, COVID-19 cases were declining, which was after the peak of the Delta variant cases in July 2022 and before the rise of cases due to the Omicron variant near the end of January 2022 according to the Indonesian National Disaster Management Authority (BNPB).¹¹

This study was ethically approved by the Medical Research and Health Ethics Commission of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, with the ethical clearance number KE/FK/1431/EC/2022.

Inclusion Criteria

The subjects from this study were post-hospitalized COVID-19-infected patients from mild to critical severity who came to the long COVID-19 clinic at UGM AH. The inclusion criteria for this study were:

1. COVID-19-confirmed patients, as evidenced by the polymerase chain reaction (PCR) test with mild, moderate, severe, or critical severity,
2. Patients who were eligible for spirometry tests and from whom a valid test result was acquired,
3. Patients were able to follow and complete the follow-up of this study, and
4. Patients were willing to become the subjects of this study.

Exclusion Criteria

The exclusion criteria of this study were patients with a history of restrictive lung diseases and patients with a history of severe chronic obstructive pulmonary disease (COPD).

Data Collection

The data collected were both primary and secondary from the patients who came to the long COVID-19 clinic at UGM AH and were recruited as subjects after signing the informed consent form. The subjects then had their spirometry test taken three months after the onset of COVID-19 symptoms. Patient-related information necessary to this study was also collected from the patients' electronic medical records during their hospital stay at UGM AH before coming to the long COVID-19 clinic. Data collected from the patients' electronic medical records were the subjects' baseline characteristics, including demographic characteristics (age and sex), comorbidities, and acute COVID-19 infection severity. Spirometry tests were taken three months after the onset of symptoms. They were performed by health workers using level 2 protective personal equipment (PPE) (scrub, gown, N95 mask, and goggles) in a special isolated room with an

exhaust fan. The spirometry test was also performed using a disposable tube to ensure patient safety.

Statistical Analyses

This study presented and analyzed spirometry parameters such as forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC, and FEF 25-75%, then compared them between results shown in the mild-moderate severity patients and severe-critical severity patients. Two types of results are presented, the mean of predicted values and the number of subjects below normal predicted values. The comparison of the mean of the predicted values between the two groups was analyzed statistically using the independent T-test or Mann-Whitney in accordance with the data distribution. Baseline characteristics and severity as a clinical predictor were analyzed using univariate logistic regression to evaluate the odds ratio (OR) of FEF 25-75% predicted value below 65%. The results are presented using a table including OR with a 95% confidence interval (95% CI).

RESULTS

Participants

Forty-two post-hospitalized COVID-19 infection patients who came to the long COVID-19 clinic at UGM

AH and were willing to be respondents were primarily included. All subjects were observed for three months since the onset of COVID-19 symptoms, but two subjects decided to withdraw from the study. Forty subjects continued the observation, 24 patients were in the mild-moderate severity group, while 16 patients were in the severe-critical severity group. Sixteen subjects were lost to follow-up when the spirometry test was due, three months after the onset of COVID-19 symptoms. Among the 16 subjects lost to follow-up, 12 were from the mild-moderate severity group, and four were from the severe-critical severity group. A total of 24 subjects were eligible for the spirometry test and had valid test results, which were distributed equally between the two severity groups of 12 subjects per group. The flow chart for the subject selection process can be seen in [Figure 1](#).

Baseline Characteristics

Baseline characteristics between the two groups were analyzed using Chi-Square, independent T, or Mann-Whitney tests in accordance with data characteristics and distribution. The results are shown in [Table 1](#).

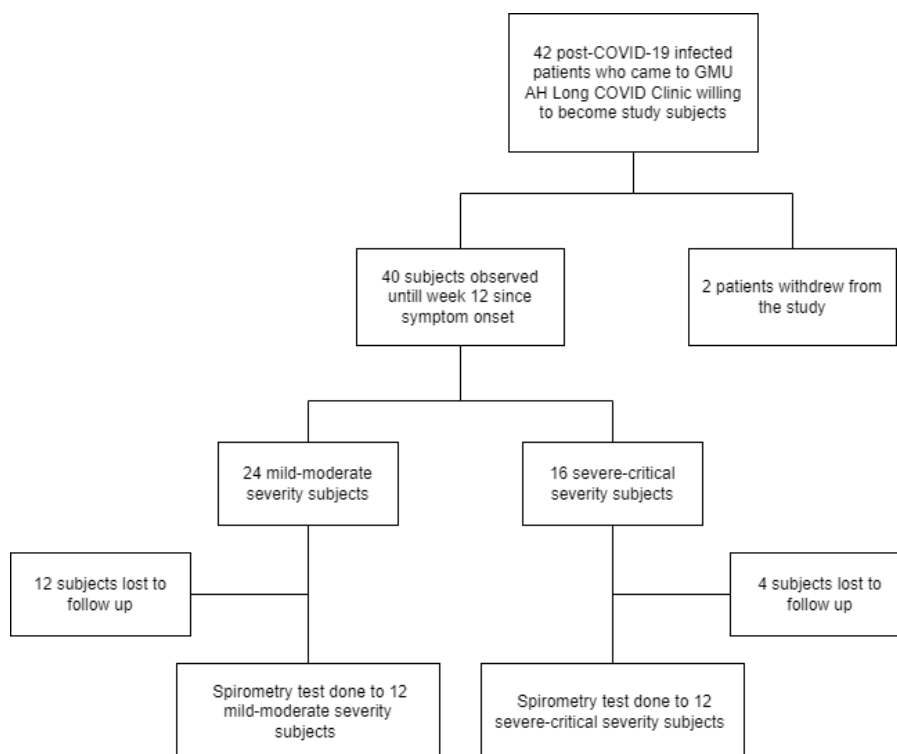


Figure 1. Flow chart of subject selection

Table 1. Subjects' baseline characteristics

Baseline Characteristic	Mild-Moderate (n = 12)		Severe-Critical (n = 12)		p-value
	Frequency	Percentage	Frequency	Percentage	
Age (years old)	64.50 (50-76)		58.50 (24-73)		0.049
Sex					
Male	8	66.67	10	84.62	0.346
Female	4	33.33	2	16.67	
Time to spirometry test since symptom onset (days)	103.083 (23.28)		104.667 (22.08)		0.866
Comorbidities					
Hypertension	4	33.33	7	58.33	-#
Diabetes mellitus	5	41.67	9	75.00	0.098
Obesity	4	33.33	8	66.67	-#
Chronic obstructive pulmonary disease	1	8.33	0	0.00	-#
Asthma	0	0.00	2	16.67	-#

n: sample size, COPD: chronic obstructive pulmonary disease. #For baseline characteristics other than diabetes mellitus, the p-value could not be calculated as the number of subjects was less than five.

A notable difference is shown in the median age between the two groups, which was proven statistically significant (p -value < 0.05), which will affect data interpretation in the discussion of this study.

Spirometry Results

Spirometry results from the tests, including FVC, FEV1, and FEF 25-75%, were compared with their predicted values and presented as percentages. The ratio of FEV1/FVC was not compared to its predicted values and is presented as a percentage. Analysis was performed by comparing the means of spirometry

parameters between the two groups. The comparison of the abnormal spirometry results did not use any statistical analyses due to the number of subjects in any of the compared groups being below five.

This study referenced the European Respiratory Society (ERS)/American Thoracic Society (ATS) 2005 for normal spirometry parameter values' cut-off; FVC and FEV1 predicted value below 80%, a ratio of FEV1/FVC below 70%, and FEF 25-75% predicted value below 65%. Spirometry results are shown in [Table 2](#).

Table 1. Spirometry results

Spirometry Parameters	Total n = 24	Mild-Moderate n = 12	Severe-Critical n = 12	p-value
n %FVC predicted <80%	8 (33.33%)	4 (33.33%)	4 (33.33%)	-
%FVC predicted	85.93 ± 13.97*	86.93 ± 13.22*	84.34 ± 15.15*	0.662 ^a
n %FEV1 predicted <80%	5 (20.83%)	1 (8.33%)	4 (33.33%)	-
%FEV1 predicted	98.83 ± 19.07*	102.99 ± 16.54*	94.67 ± 21.19*	0.295 ^a
n FEV1/FVC <70%	2 (8.33%)	0 (0.00%)	2 (16.67%)	-
%FEV1/FVC	87.83 (51.74 – 100) [#]	85.07 (70.64 – 100) [#]	90.21 (51.74 – 96.89) [#]	0.729 ^b
n %FEF 25-75% predicted <65%	5 (20.83%)	3 (25.00%)	2 (16.67%)	-
%FEF 25-75% predicted	96.18 ± 44.36*	98.29 ± 49.87*	94.07 ± 40.22*	0.822 ^a

n: sample size, FVC: forced vital capacity, FEV1: forced expiratory volume in the 1st second, FEF: forced expiratory flow, a: analyzed by independent T-test, b: analyzed by Mann-Whitney test, data presented in the form of frequency and percentage, mean, and standard deviation (*), as well as median and range (#)

This study did find several subjects' spirometry results below normal, but the means of each parameter were within normal ranges in each group. This study showed no significant differences in the comparison of means between the two groups.

Abnormal FEF 25-75% Predicted Value Clinical Predictors

A univariate logistic regression test was performed to calculate the adjusted OR of clinical predictors to an abnormal FEF 25-75% predicted value

with a 95% CI. The clinical predictors tested were age, sex, comorbidity (diabetes mellitus/DM), and acute COVID-19 infection severity. Other comorbidities mentioned in this study, such as hypertension, obesity, COPD, and asthma, were not tested using the logistic regression because the number of subjects in any of the compared groups was below five.

Table 3 shows the adjusted OR with the clinical predictors' 95% CI. This study showed no significant OR to an abnormal FEF 25-75% predicted value from the clinical predictors.

Table 2. Adjusted OR of clinical predictors to an abnormal FEF 25-75% predicted value

Clinical Predictors	Adjusted OR with 95% CI
Age	1.07 (0.94-1.21)
Sex (Male)	0.78 (0.05-11.14)
Diabetes Mellitus	3.92 (0.34-45.49)
Severity (Severe-Critical)	0.62 (0.04-8.97)

DISCUSSION

This study did not fulfil its sample size requirements, which were 70 subjects in total or 35 subjects for each severity group. This study was conducted when COVID-19 cases were declining worldwide, including in Indonesia at the mid to end of 2021. According to the data from BNPB, the peak of the Delta variant COVID-19 cases in Indonesia happened in July 2021 and declined in the following months, while the increase of COVID-19 cases due to the Omicron variant happened at the end of January 2022 and peaked in February 2022.¹¹ This study started at the end of August 2021 until the end of January 2022, coinciding with the low number of COVID-19 cases in Indonesia. This resulted in the low number of patients coming to the long COVID-19 clinic at UGM AH after being hospitalized and caused the low number of subjects in this study. This study considered that the low number of subjects would significantly affect the results and interpretation of this study.

Comparison of Spirometry Results between Two Severity Groups

This study showed no significant differences between the two groups' means/median of spirometry parameters. The results were also within normal ranges (Table 2). This might be due to the small sample size of this study. However, Patria and Sabirin (2021) also showed no significant mean/median differences between the two severity groups.¹² It is also reported that the means and median of the spirometry parameters were within normal ranges. Liao, *et al.* (2020) conducted a study comparing spirometry results of severe and non-severe COVID-19 infection three months after discharge and reported that the difference in the means of

spirometry parameters between severe and non-severe groups was insignificant.¹³

This study showed restrictive pattern defects were more frequent than obstructive patterns. Previous studies have also reported that restrictive patterns dominated long COVID-19 patients.^{14,15} One observational study involving thirty-four days follow-up of 146 patients recovered from mild COVID-19 showed 20% of patients with a restrictive pattern and only 3% of patients with an obstructive pattern (defined by FEV1/FVC ratio below the lower limit of normal/LLN).¹⁵ This might be due to several factors, such as the angiotensin-converting enzyme-2 (ACE-2) receptor being expressed more in alveolar epithelial cells than the airways and reduced surfactant production from type 2 pneumocytes expressing ACE-2 receptors. ACE-2 receptor is the entry gate for the COVID-19 virus. Hence, viral replication occurs in the cells with ACE-2 receptors. The alveolar cells will be destroyed and produce fibrotic tissue. This fibrotic process causes restrictive lung in COVID-19 patients.^{12,16}

Obstructive pattern defects were also found in this study. This may occur mainly through small airway involvement, as explained by Elicker, where bronchioles may be affected, causing concentric fibrosis and an obstructive pattern defect.⁸

Several subjects in this study also showed a decrease in the FEF 25-75% predicted value. While a decrease in FEF 25-75% predicted value is common in asthmatic patients, other causes may be due to pollution and work environment hazards, smoking, early stages of COPD, and other unknown factors.¹⁶ This study showed a decrease in the predicted FEF 25-75% value in five subjects. One of them had a history of COPD, belonging to the mild-moderate severity group, while the other four did not have a history of either asthma, COPD, or smoking. Interestingly, two subjects in the severe-critical group with a below-normal FEV1/FVC ratio also had a below-normal FEF 25-75% predicted value. It can be inferred that the obstructive pattern defect in these two patients may be due to small airway disease. Notably, these two patients had no asthma, COPD, or smoking history.

Small airway disease as a long-term effect of COVID-19 can happen due to the abundance of ACE-2 and transmembrane serine protease 2 (TMPRSS-2) receptors in transient secretory cells in the subsegmental bronchus.¹⁷ As the subsegmental bronchus is a part of the small airway, any damage causing fibrosis or narrowing may cause a decrease in FEF 25-75% results. In addition, another proposed mechanism of shortness of breath in post-COVID-19 patients is the increased expression of the chemokine receptor C-X-C chemokine

receptor type 6 (CXCR6) and the adhesion molecule P-selectin glycoprotein ligand-1 (PSGL1) in monocyte.¹⁸

OR of Clinical Predictors to an Abnormal FEF 25-75% Predicted Values

This study did not show any significant adjusted OR between the clinical predictors such as age, sex, comorbidity (DM), and severity to an abnormal FEF 25-75% predicted value. A previous study by Yazji, *et al.* (2022) showed the unadjusted OR for baseline characteristics with any lung function abnormality to be significant in several clinical predictors, such as Intensive Care Unit (ICU) admission, elevated D-dimer level (above 250 ng/L), hypertension, and DM.¹⁹ This study might have shown no significant OR due to the small sample size.

Study Limitations

This study has several limitations. First, this study did not have any spirometry data of the subjects prior to COVID-19 infection. Therefore, it is unclear whether the abnormalities in this study were caused by COVID-19 or had happened before. Second, the time of spirometry test since the onset of symptoms has a wide standard deviation and cannot accurately characterize the patients as homogenous (even though the difference between the two groups is insignificant). Third, this study did not assess lung diffusing capabilities and could not describe any diffusion abnormalities. Fourth, this study is inadequate to describe the long-term lung function effects of COVID-19 in young adults due to only having one subject below 50 years old.

In addition to spirometry, small airway disease can be detected using a computed tomography (CT) scan or additional non-conventional pulmonary function tests (PFT), such as forced oscillation technique (FOT) and multiple breathing washout (MBW). FOT and MBW, in one case report, showed increased detection of small airway disease in post-COVID-19 patients.²⁰ A normal PFT result does not necessarily indicate the absence of small airway disease because of its low specificity.¹⁰ Thus, future research should consider non-conventional PFT or additional imaging, such as CT scans, to improve the detection of small airway disease in post-COVID-19 patients.

CONCLUSION

This study showed that a small proportion of post-COVID-19 syndrome patients had small airway disease three months after the onset of symptoms of mild-moderate and severe-critical groups. However, the two severity groups showed no statistical difference in small airway function.

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Conflict of Interest

The authors declared there is no conflict of interest.

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Authors' Contributions

Conceptualizing and designing the study: SS. Collecting data: MK, HAR. Interpreting results: MK, SS, HAR, SHR. Preparing manuscript: MK, SS, RMI, RMS, RAW. Revising manuscript: HAR, SS, RMI, RMS, RAW, SHR.

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