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FeNO as a biomarker of interstitial and fibrotic pulmonary sequelae in patients admitted for severe SARS-CoV-2 pneumonia

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Pulmonary fibrosis after severe SARS-CoV-2 pneumonia is a major sequela in surviving patients which requires evaluation. Fractional exhaled nitric oxide (FeNO) is a marker of airway inflammation, easy to obtain and available in most functional testing laboratories of pulmonology services. Our objective was to evaluate the capacity of FeNO as a biomarker of interstitial and fibrotic pulmonary sequelae in patients admitted for severe SARS-CoV-2 pneumonia. We recruited 335 patients admitted for severe pneumonia secondary to SARS-CoV-2 who were being followed up at the Diffuse Interstitial Lung Disease unit at Hospital Universitario Marqués de Valdecilla. FeNO levels were higher in patients with fibrotic interstitial sequelae: mean 24.3 vs. 19.8 ppbs, $p = 0.002$, with an area under the curve (AUC) of 0.63; 95% confidence interval (CI) 0.57–0.69 and an optimal cut-off point of 11 ppb maximizing the weighted combination of Sensitivity and specificity. FeNO ranked 6th among the 18 variables studied using various methods (forward selection, backward elimination, and stepwise regression) in evaluating the predictive ability for fibrotic interstitial sequelae, and it was the 5 th most predictive variable after using the cut-off point of 11 ppb. The joint predictive ability of the overall model with the 6 more predictive variables was higher than 0.8: AUC (Use of systemic corticosteroids + peak C-reactive Protein at admission + Age + Endotracheal intubation + Diffusing Capacity for CO (DLCO) + FeNO as quantitative continuous) = 0.81; 95%CI (0.77–0.86). AUC of the same model with FeNO as dichotomous (11 ppb cut-off point) = 0.82; 95%CI (0.78–0.87). Our study shows an increase in FeNO in patients who, after admission for severe SARS-CoV-2 pneumonia, present fibrotic interstitial sequelae at the three-month follow-up, as one of the different predictive variables related to the presence of these sequelae.

Keywords FeNO, COVID-19, SARS-CoV-2, Sequeale, ILD, Pulmonary fibrosis

Since the declaration of theSARS-CoV-2 pandemic by the World Health Organization (WHO) in March 2020, millions of people have been infected, putting a strain on health systems worldwide[1]. In its most severe form, this viral infection can lead to adult respiratory distress syndrome (ARDS) with severe respiratory failure. Despite the development of vaccines and specific treatments that have improved patient survival and reduced the progression of the disease, certain patients still develop a severe disease[2–4].

The presence of long-term sequelae significantly impairs the quality of life of those affected. Although these sequelae do not depend exclusively on the severity of the disease, a close monitoring of patients who have overcome a severe disease is important for early detection[5–7].

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Among the most important sequelae following severe SARS-CoV-2 is the development of pulmonary fibrosis or interstitial lesions in the lung parenchyma[8]. In the different systematic reviews published to date, the data on the prevalence of fibrotic sequelae in the different studies are highly heterogeneous[9,10].

The different scientific societies of respiratory pathology have developed protocols to follow-up patients admitted for SARS-CoV-2 pneumonia, in order to monitor the presence of these sequelae. Most of these protocols are based on the results obtained through imaging tests and forced spirometry and diffusion. In recent years, many studies have research other types of biomarkers that could assist with decision-making, e.g., to assess when to order radiation-emitting tests such as chest computed tomography (CT), or to evaluate the presence of pulmonary fibrosis[11–14].

Fractional exhaled nitric oxide (FeNO) is a technique used to detect the presence of airway inflammation, available in most respiratory function testing laboratories. FeNO is noninvasive, inexpensive, safe, and easy to perform. It is a basic test used for the diagnosis and management of bronchial asthma[15–17]. In the past, it has been studied for the assessment of different interstitial lung diseases[18]. Recently, several studies have evaluated its usefulness both for the measurement of the severity of an acute SARS-CoV-2 disease and as a biomarker of sequelae at the pulmonary level after the disease[19–21].

The hypothesis that FeNO may be a marker of interest in the presence of fibrotic interstitial sequelae following severe SARS-CoV-2 pneumonia is that most cells in the respiratory tract can produce nitric oxide (NO) including pneumocytes I and II, bronchial epithelium, endothelium, smooth muscle cells and alveolar immune cells (macrophages, eosinophils and neutrophils)[22]. Inducible nitric oxide synthase (iNOS) is considered to contribute to more than 60% of NO production in the airways and is generally activated by immune and epithelial cells, triggered by inflammatory or infectious processes. In addition, it has been extensively studied as a marker in different interstitial affections, even considering its usefulness to differentiate between them[23,24]. However, it should also be noted that angiotensin-converting enzyme 2 (ACE2) decreases iNOS signaling and is the gateway for the SARS-CoV-2 virus to enter respiratory epithelial cells, which raises the hypothesis of a negative correlation between COVID-19 infection and the production of NO in the airways and, therefore, the FeNO values[25–27].

Although further studies are needed, FeNO measurement could be implemented in the follow-up of patients with severe SARS-CoV-2 pneumonia for decision making regarding the request of a HRCT scan for sequelae assessment. The aim of our study was to evaluate the ability of FeNO as a biomarker in a screening context of interstitial/fibrotic pulmonary sequelae in patients who were admitted for severe SARS-CoV-2 pneumonia.

Materials and methods

Study population and design

A cross-sectional study with prospective consecutive recruitment of patients admitted to hospital for severe SARS-CoV-2 pneumonia. The study took place between June 2020 and March 2022 at Hospital Universitario Marqués de Valdecilla, a regional and national reference center, and part of the European Reference Network on rare respiratory diseases (ERN-lung) for diffuse interstitial lung diseases. The study was approved by the Ethics Committee for Research on Medicines and Medical Devices of Cantabria (internal code: 2020.480). All participants gave written informed consent to participate in the study. It was protocolized the recruitment of at least 300 patients to ensure an statistical power > 80% to detect as statistically significant mean differences of FeNO levels ≥ 5 ppb by using a two-sided Student's t-test with an alpha error of 5%, and a standard deviation (SD) of 14 pbb.

The exclusion criteria were: (1) comorbidities that could influence FeNO values: asthma, allergic diseases, obstructive pulmonary pathology, pulmonary hypertension, pre-existing interstitial disease, chronic rhinosinusitis with or without nasal polyps and peripheral eosinophilia; (2) patients with pre-admission treatment with oral or inhaled corticosteroids and phosphodiesterase-5 inhibitors; (3) inability to perform respiratory function tests correctly; (4) positive bronchodilator test in respiratory function tests.

In total, 436 patients were recruited who were admitted for pneumonia with oxygen requirements secondary to SARS-CoV-2 and who were being followed up at the diffuse interstitial lung diseases consultations of the Pneumology Department. After verification of the inclusion criteria, 101 patients were excluded. A flow-chart of the patients included in the study is shown in Fig. 1.

Measurements

During the consultation different assessments were made pulmonary function tests (PFTs) including: forced spirometry, diffusion, plethysmography and FeNO. High resolution computed axial tomography (HRCT) and control laboratory tests with multiple parameters, highlighting some related to the severity of the disease on admission, such as D-dimer (DD), ferritin or lactate dehydrogenase (LDH). This was in accordance with the consensus document of the Spanish Society of Respiratory Pathology (SEPAR) for post-COVID clinical follow-up[11]. This consultation also included the patient's demographic and clinical data, which was gathered in a database. The clinical data considered severity criteria during admission, both analytical and by scales, days of admission, pharmacological treatments received as well as respiratory therapies. Severity during admission was measured using the COVIDGRAM scale, a scale designed in China based on 10 variables (radiological alteration, age, hemoptysis, dyspnea, confusion, number of comorbidities, history of cancer, neutrophil/lymphocyte ratio, lactate dehydrogenase and direct bilirubin) according to the results, patients are classified as low, moderate and high risk. The presence of a high-risk score on this scale confers a 59.3% probability of admission to the ICU or death from SARS-CoV-2 infection. This scale was validated for our population in a study by Armiñanzas et al.[28].

The assessment of radiological involvement and the presence of fibrotic interstitial involvement was performed by experienced radiologists of chest radiography. All lung interstitial changes described in the literature were

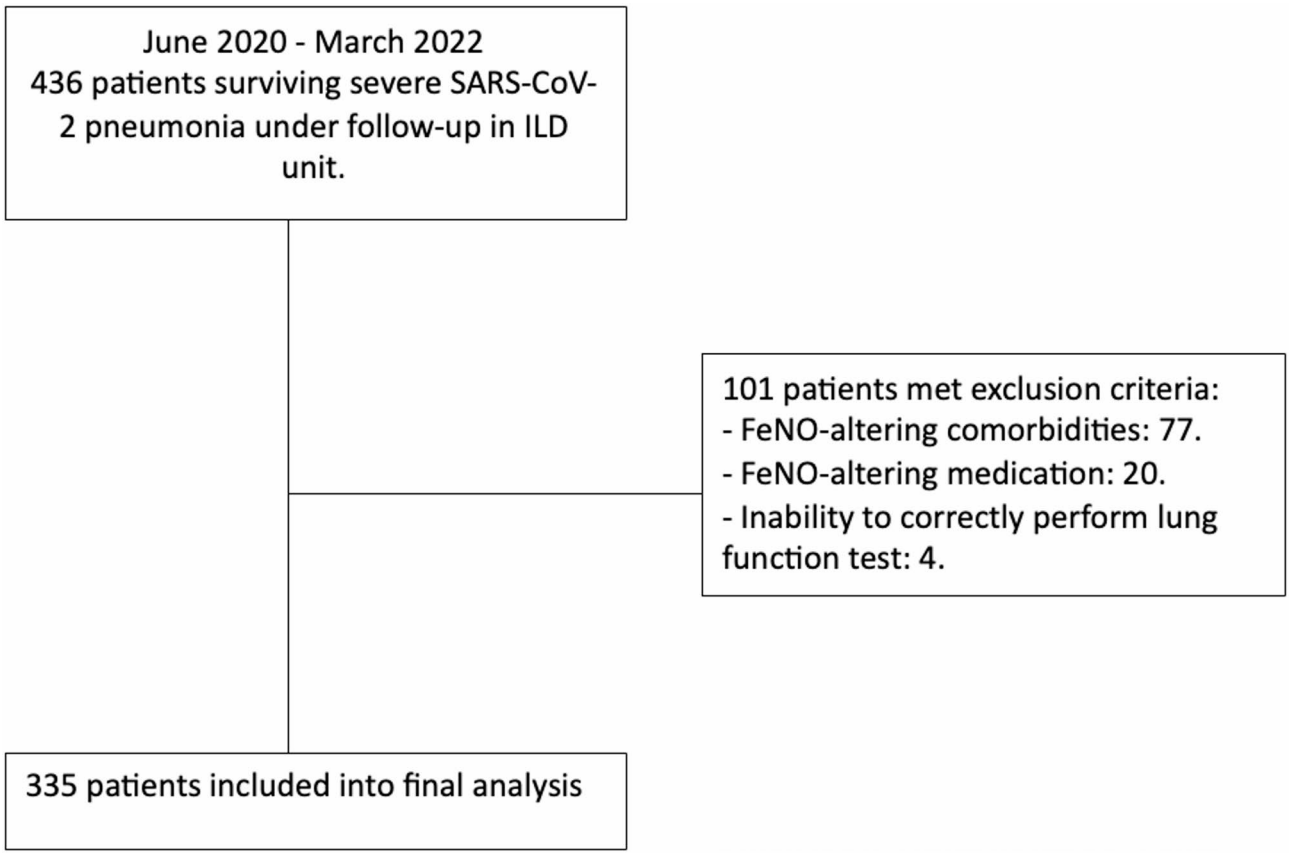

Fig. 1. Flow-chart of patients included in the study.

included, including ground-glass opacities, honeycombing, fibrotic-like changes, reticulation, and interlobular septal thickening[29].

Part of the samples and patient data included in this study were provided by the Valdecilla Biobank (PT17/0015/0019), integrated in the national biobank network, with the approval of the Ethics and Scientific Committees, and have been processed following standardized procedures.

The following pulmonary function test (PFTs) were carried out in accordance with ATS standards[30]: forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), diffusion value of carbon monoxide (DLCO). These were always performed after evaluation of FeNO in a Jaeger-Carefusion MasterScreem combi model that was calibrated daily.

FeNO was performed in NIOX VERO ® according to ATS/ERS recommendations for measurement in adults[31]. Prior to spirometry in accordance with these regulations, patients were prohibited from strenuous physical exercise, smoking, and consuming certain foods and beverages.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the patients in each group. Continuous variables were expressed as the mean and SD in the case of normally distributed variables, or as median and interquartile range (IQR) in the case of variables distributed assmimetrically. Categorical variables were presented as percentages. For continuous variables the difference between groups was assessed by the Student's t-test or the Mann-Whitney U-test, and by the Chi-square test for the comparative analysis of proportions.

Variables potentially associated with the presence of fibrotic interstitial sequelae were predefined on the three-month HRCT, yielding 10 quantitative variables (including FeNO) and eight qualitative variables (Table S1).

To identify those variables most predictive of the risk of fibrotic interstitial sequelae in the HRCT at three months, all these variables were introduced into predictive models under three different strategies: sequential inclusion method (Forward selection); sequential exclusion method (Backward elimination), stepwise regression (Stepwise regression); obtaining the most predictive final models in each of these strategies.

To compare the predictive value of the more predictive quantitative variables obtained, receiver-operating characteristic (ROC) curves were constructed and the area under the curve (AUC) was determined, with the presence of absence of fibrotic interstitial sequelae on HRCT at three months as the outcome.

With the absence of a cut-off point for FeNO in this type of patient, the optimal cut-off points for FeNO levels were calculated considering the values that maximized the weighted combination of sensitivity (Se) and specificity (Sp) (i.e. that maximized the Youden index) for a ratio false-negatives cost/false-positives = 1 and 1.5

and for the prevalence of fibrotic interstitial sequelae on HRCT at three months in our sample. These cut-off points were 19.5 ppb and 11 ppb respectively. In addition, a cut-off point = 25 ppb was also considered as it is the cut-off point where the American Thorax Society (ATS) committee for the interpretation of FeNO levels indicates the possible presence of airway inflammation[32]. Based on these three cut-off points, the main parameters of diagnostic validity were estimated: Sensitivity (Se), Specificity (Sp), positive predictive value (PV+), negative predictive value (PV-) and likelihood ratios. The 95% confidence intervals (95% CI) were calculated for all the estimations using Wilson's method.

The cut-off point of 11 ppb, in addition to being supported by our results, coincides with that of other similar studies[33] and was therefore introduced in the final model of predictor variables. Lastly, an overall AUC was estimated by using a whole regression model with the final most predicted variables.

Data documentation and statistical analyses were performed with SPSS version 22.0 (SPSS Inc., Chicago, IL, EE. UU.).

Results

Study population according to the presence or absence of fibrotic interstitial sequelae in the HRCT at three months

The mean age of the 335 patients included in the study was 62.8 ± 12.7 years, including 65.7% men ($n = 220$). The prevalence of fibrotic interstitial sequelae in the HRCT at three months was 54.63% ($N = 183/335$). When dividing the patients according to the presence of fibrotic interstitial sequelae at the three-month control CT, it was observed that the patients with sequelae were older ($p < 0.001$), and most of them were men. They presented greater severity during admission with a higher COVIDGRAM scale ($p < 0.001$), with higher analytical markers of inflammation, highlighting higher peak ferritin, peak DD, peak LDH and peak CRP during admission ($p < 0.05$ in all of them). They also had a higher use of high-flow nasal therapy (HFNT) ($p = 0.007$) and the need for endotracheal intubation (ETI) ($p < 0.001$). They had more frequently received anti-inflammatory treatments: corticosteroids ($p < 0.001$) and tocilizumab ($p = 0.004$). They presented a greater number of days of admission ($p < 0.001$). At the three-month follow-up, in terms of respiratory function tests, patients with sequelae had a greater loss of DLCO ($p < 0.001$) a greater FeNO ($p = 0.002$) and at the analytical level they presented a greater DD ($p = 0.024$) (See Table 1).

FeNO as a predictive variable for risk of fibrotic interstitial sequelae in HRCT at three months: ROC and regression (forward, backward, Stepwise) analysis

FeNO levels were higher in patients with fibrotic interstitial sequelae on HRCT: median = 24.3, IQR (22.3–26.2) in patients with sequelae vs. 19.8 (17.7–21.8) in patients without (See Fig. 2. Table 2).

Of the 18 variables entered (including the 10 quantitatives and 8 qualitatives) (See Table S1) FeNO ranked 6th in predictive ability in all approaches (forward selection, backward elimination, and stepwise regression) (See Table S2) and 4th among the quantitative variables behind peak CRP at admission, age and DLCO, with an AUC = 0.634; 95%CI (0.57–0.69) (See Table S3. Figure 3).

The main parameters of diagnostic validity for the three FeNO cut-off points are presented in Table 2. The cut-off point = 11 ppb was associated to a Se = 93.44%; 95%CI (88.89–96.21), and a Sp = 16.45%; 95%CI (11.40-23.15), a PV + = 57.38% and a PV- = 67.57%.

When FeNO was introduced as a dichotomous variable with the optimal cut-off of 11, FeNO was the 5th most predictive variable, thus moving up one place (its predictive ability improved) (See Table S4). The complete regression results are presented in Table S5.

Predictive ability of the final model

The joint predictive ability of the joint overall model with the 6 more predictive variables (Corticosteroids + peak CRP at admission + Age + IOT + DLCO + FeNO as quantitative continuous) was greater than 0.8: AUC = 0.814; 95%CI (0.769–0.860). When replacing this FeNO with its dichotomous equivalent based on the 11 ppb cut-off point, the predictive ability of the model was higher with an overall AUC = 0.822; 95%CI (0.777–0.869) (See Table 3).

Discussion

This study presents our experience in the follow-up of patients after severe SARS-CoV-2 pneumonia. Several variables have been related to the presence of fibrotic interstitial sequelae at the three-month follow-up. The most important variables are related to the severity of pneumonia during admission and the age of the patients, as previously described in the literature[7,25].

A relevant finding in our sample was that patients with the presence of fibrotic interstitial sequelae at the three-month HRCT scan had a statistically significantly higher FeNO. The results obtained for FeNO associated with SARS-CoV-2 infection, whether for the purpose of assessing the severity of the infection or as a marker of follow-up and sequelae after infection, are controversial. Most research examining FeNO in post-COVID patients consists of studies comparing FeNO in patients who have suffered a SARS-CoV-2 infection with that of healthy controls. In Hua-Huy et al. study, the largest cohort in terms of number of patients to date ($n = 169$), FeNO and CaNO were assessed at least six weeks after surviving a SARS-CoV-2 infection, and the severity of the patients was determined by the lung surface area affected at the diagnostic HRCT scan[26]. These authors observed that patients with more severe HRCT involvement had statistically significantly higher CaNO, yet no significant differences in FeNO. In the study by Cameli et al., which is the most similar to ours, the cost-effectiveness of FeNO as a biomarker of fibrotic-interstitial sequelae was examined, finding an increase in CaNO in patients with sequelae, yet no significant alterations in other FeNO parameters[19]. The principal problem with CaNO is that

	Global ($n=335$)	HRCT scan with fibrotic interstitial sequelae ($n=183$)	HRCT scan without fibrotic interstitial sequelae ($n=152$)	p value
Age, mean ± SD	**62.8 ± 12.7**	**66.1 ± 1.2**	**58.9 ± 13.2**	***< 0.001***
Male sex, n (%)	**220 (65.7%)**	**134 (72.3%)**	**86 (56,6)**	***0.001***
COVIDGRAM (high), n (%)	**107 (31.9%)**	**76 (41.5%)**	**31 (20.4%)**	***< 0.001***
HTN, n (%)	**143 (42.7%)**	**92 (50.3%)**	**51 (33.6%)**	***0.002***
DM type 2, n (%)	**57 (17%)**	**38 (20.8%)**	**19 (12.5%)**	***0.045***
Ischemic heart disease, n (%)	26 (7.8%)	16 (8.7%)	10 (6.6%)	*0.461*
Immunosuppression, n (%)	18 (5.4%)	10 (5.5%)	8 (5.3%)	*0.935*
CKD, n (%)	16 (4.8%)	12 (6.6%)	4 (2.6%)	*0.093*
Onco-hematological pathology, n (%)	14 (4.2%)	8 (4.4%)	6 (3.9%)	*0.847*
Smoking, n (%)	46 (13.7%)	23 (12.6%)	23 (15.1%)	*0.497*
Peak DD on admission (ng/ml), mean (95%CI)	**5382.3 (4054.6-6170.01)**	**7358.9 (5209.7-9508.08)**	**3002.6 (1706.3-4298.9)**	***< 0.001***
Peak Ferritin on admission (ng/ml), mean (95%CI)	**1034.5 (921.04-1148.08)**	**1228.8 (1054.9-1402.8)**	**800.6 (671.1-930.1)**	***0.002***
Peak LDH on admission (U/l), mean (95%CI)	**376.8 (358.6-395.02)**	**417.3 (390.8-443.8)**	**328 (305.6-350.3)**	***< 0.001***
Peak CRP on admission (mg/dl) mean (95%CI)	**13.2 (12.2–14.1)**	**16.04 (14.7–17.3)**	**9.8 (8.6–10.9)**	***< 0.001***
Corticosteroids, n (%)	**210 (62.7%)**	**145 (79.2%)**	**65 (42.8%)**	***< 0.001***
Tocilizumab, n (%)	**70 (20.9%)**	**49 (26.8%)**	**21 (13.8%)**	***0.004***
High-flow nasal therapy (HFNT), n (%)	**111 (33.1%)**	**83 (45.4%)**	**28 (18.4%)**	***< 0.001***
Endotracheal intubation (ETI), n (%)	**101 (30.1%)**	**80 (43.7%)**	**21 (13.8%)**	***< 0.001***
BMI, mean (SD)	29.6 ± 4.7	29.1 ± 3.9	30.2 ± 5.5	*0.137*
Days of hospital admission, mean (95%CI)	**16.6 (14.8–18.4)**	**21.3 (18.6–24.1)**	**10.9 (9.3–12.5)**	***< 0.001***
DLCO (%) at three months at three months, mean (95%CI)	**81.5 (79.5–83.4)**	**77.1 (74.5–79.6)**	**86.7 (83.9–89.6)**	***< 0.001***
FVC (%) at three months, mean (95%CI)	100.01 (98.1-101.9)	98.2 (95.8-100.7)	102.1 (99.1-105.04)	*0.224*
FeNO (ppb) mean (95%CI)	**22.2 (20.8–23.7)**	**24.3 (22.3–26.2)**	**19.8 (17.7–21.8)**	***0.002***
DD at three months (ng/ml), mean (95%CI)	**510.8 (453.9-567.7)**	**567.6 (477.5-657.6)**	**442.5 (380.2-504.8)**	***0.024***
Ferritin at three months (ng/ml), mean (95%CI)	144.1 (125.8-162.3)	156,7 (131.6-181.7)	128.9 (102.3-155.5)	*0.247*
LDH at three months (U/l), mean (95%CI)	210.4 (203.5-217.2)	210.02 (204.7-215.2)	210.8 (197.01–224.7)	*0.247*

Table 1. Demographic, functional and severity characteristics of the study population divided by sequelae. HRCT = High resolution computed axial tomography, HTN = Hypertension, DM = Diabetes Mellitus, CKD = Chronic Kidney Disease, DD = D-Dimer, LDH = Lactate Dehydrogenase, CRP = C-reactive Protein, BMI = Body Mass Index, DLCO = Diffusing Capacity for CO, FVC = Forced Vital Capacity, IQR = Interquartile range, SD = Standard deviation. Significant values are in bold. Significant values are in italics.

its estimation requires three exhalations at three different flow rates following a conventional linear regression model. The procedure is complex and challenging to complete compared to the simplicity of FeNO. In addition, the study consisted of a small sample of 20 patients who had been admitted for their SARS-CoV-2 infection. Radiological follow-up as in our study was performed on average three months after admission. These patients were compared with 22 healthy controls in which, again, CaNO was statistically significantly superior. Apart from a smaller sample size than ours, the patients in this study would have been less severe during admission as there is a lower % of ICU admission (30% vs. 20%).

In the study by Maniscalco et al., no significant difference was observed between FeNO of patients who had suffered a SARS-CoV-2 infection and healthy controls[20]. They also reported that patients who had received systemic corticosteroids for their infection had significantly lower FeNO than those without. Again, this is a study with a small cohort of patients ($n=68$), where the severity of their infection is unknown, and, as the authors themselves indicate in the discussion, they had a lower percentage of interstitial sequelae than the study by Cameli et al., yet with no indication as to what percentage this was.

Despite the contradictory results to date, in our study population the results seem conclusive. We found a significant increase in FeNO levels in patients with HRCT sequelae. Moreover, our cut-off point of 11 ppb, supported by statistical criteria, is similar to that found in other articles studying FeNO in pulmonary involvement secondary to SARS-CoV-2 infection[33].

This cut-off point in our sample is associated with a high sensitivity: 93.44%, therefore the % of false negatives is only 6.56%. This means that only 6.56% of patients with sequelae on HRCT had FeNO levels below 11 ppb. Furthermore, the negative predictive value was 67.57%, i.e. if a patient in our sample had an FeNO level of less than 11 ppb, the probability of having an HRCT scan without lesions is 67.57%.

In addition, our study presents novel findings. We performed predictive models using three inclusion methods (forward selection, backward elimination, and stepwise regression). This allowed us to assess the predictive ability of the presence of sequelae at the pulmonary level of the different related variables shown in Table 1. With this model we observed that FeNO is one of the variables that most predicts the presence of sequelae, and that if we take into account the cut-off point of 11 ppb, it does so with even greater predictive ability. We found it to be among the four most predictive quantitative variables out of the ten we considered, and among the top six if

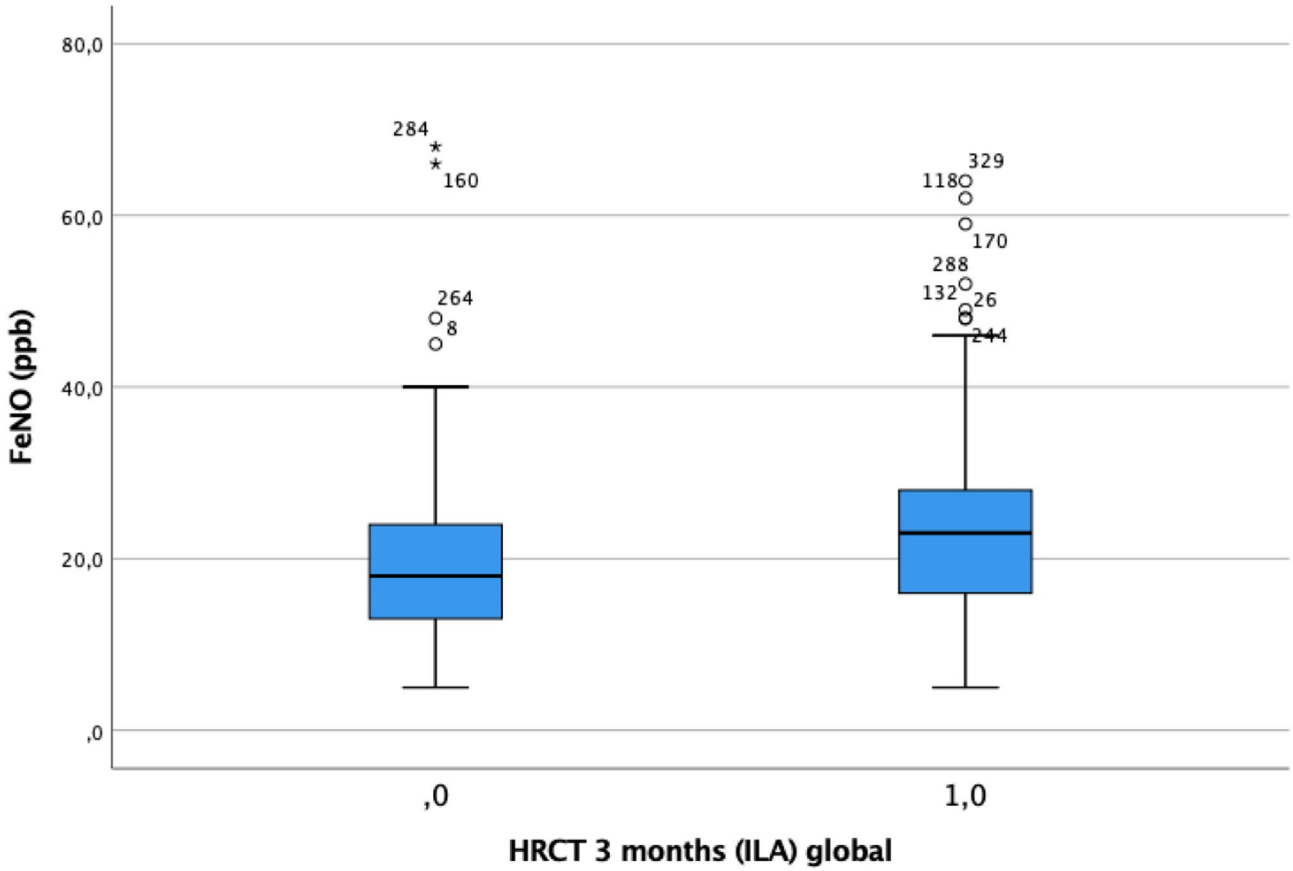

Fig. 2. FeNO levels according to the presence of fibrotic interstitial sequelae in the HRCT at three months.

Cut-off	Se(%)	Sp(%)	J(%)	LR+	1/LR-	PV+(%)	PV-(%)
≥ 11	93.4	16.4	9.9	1.12	2.51	57.4	67.6
≥ 19.5	65.0	59.2	24.2	1.59	1.69	65.7	58.4
≥ 25	42.6	75.7	18.3	1.75	1.32	67.8	52.3

Table 2. Main parameters of diagnostic validity for the three FeNO cut-off points in relation to fibrotic interstitial sequelae on HRCT at three months. FeNO = Fractional exhaled nitric oxide. HRCT = High resolution computed tomography. Cut-off = optimal cut-off points for FeNO (ppb) obtained according to the Youden index (11 ppb and 19.5 ppb) or according to the literature (25 ppb) Se = sensitivity Sp = specificity J = Youden index, LR = Likelihood ratios PV = predictive values for sample prevalence of fibrotic interstitial sequelae on HRCT at three months (54.63%).

we consider the 18 variables (ten quantitative and eight qualitative) only to be behind variables already widely studied such as age and indicators of severity of SARS-CoV-2 pneumonia during admission[7,25]. Finally, although none of the six final predictor variables obtained an AUC higher than 0.8, when all six are taken together they are associated with an overall predictive ability higher than 0.8, a value considered in epidemiological methodology as a good predictive ability.

All these results on FeNO would indicate the usefulness of its implementation in clinical practice in the follow-up of patients after severe SARS-CoV-2 pneumonia, and could be of great help in making decisions regarding the performance of a HRCT to assess the presence of fibrotic interstitial sequelae, with 11 ppb being the optimal cut-off point.

In the past, both FeNO and CaNO have proven to be effective markers to indicate the presence of pulmonary fibrosis of different causes. In addition, they have also been linked to the progression, severity and prognosis of ILD. Their relationship with other respiratory function parameters such as FVC, FEV1, TLC and 6 min test is of interest and has been reported in the literature[34–36]. In our study, a cross-sectional study, we looked for the possibility that FeNO was a marker of fibrotic sequelae in interstitial lung fibrosis in the third month after admission for severe post-SARS-CoV-2 pneumonia. As previously indicated, CaNO was not taken into account due to the greater technical difficulty in obtaining it. A simple and reproducible marker was sought in any

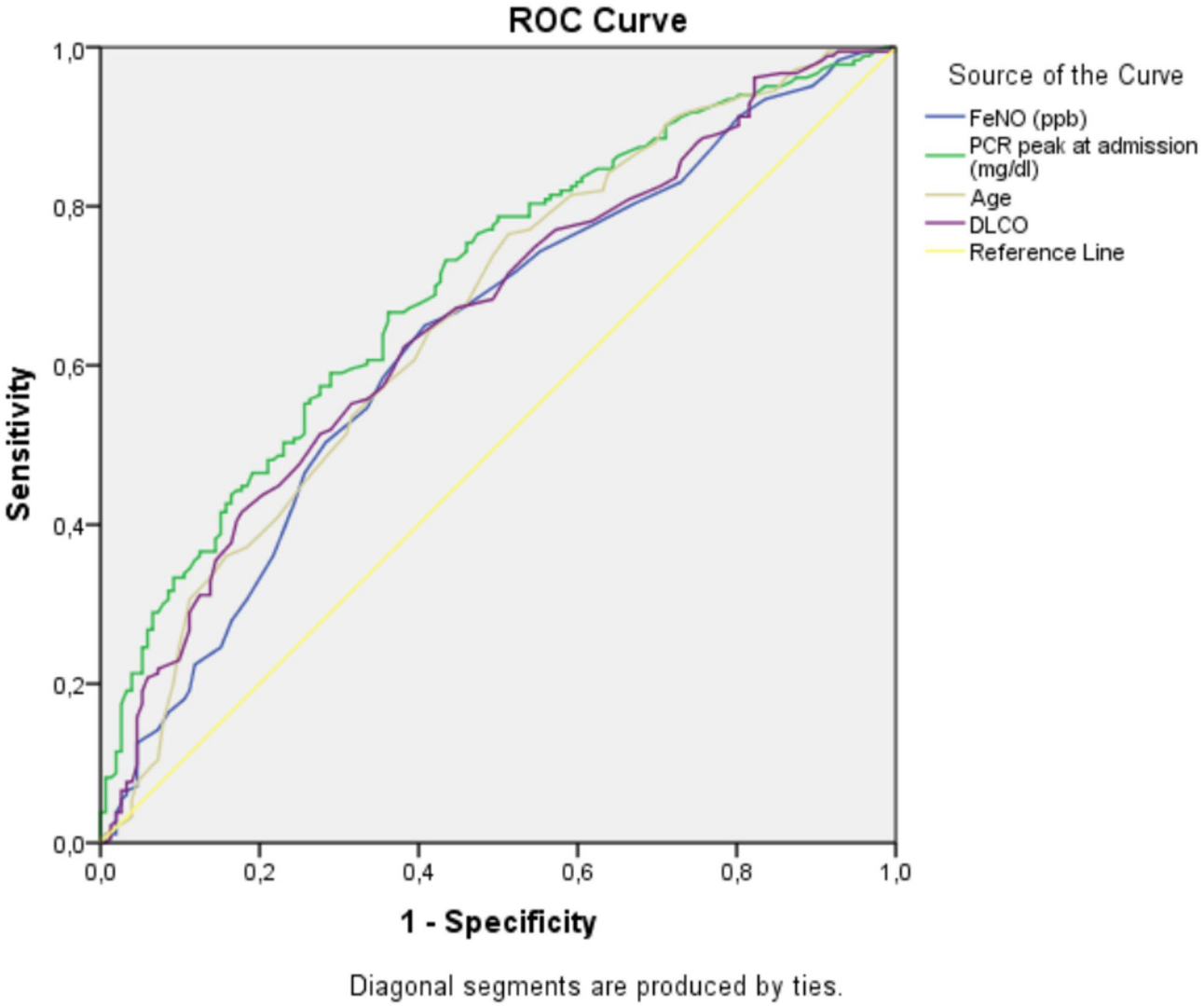

Fig. 3. ROC curves for the most predictive quantitative variables in relation to existence of fibrotic interstitial sequelae on HRCT at three months.

	AUC*	(CI	95%)
Corticosteroids + Peak CRP admission + Age + ETI + DLCO + FeNO ppb (continuous)	0.814	0.769	0.860
Corticosteroids + Peak CRP admission + Age + ETI + DLCO + FeNO (11ppb cut-off point)	0.822	0.777	0.866

Table 3. AUCs and their 95% confidence intervals (CIs) estimated through regression models for the final combination of six variables in relation to fibrotic interstitial sequelae on HRCT at three months. HRCT = High resolution computed tomography, AUC = area under the ROC curve, CRP = C-reactive Protein, ETI = Endotracheal intubation, DLCO = Diffusing Capacity for CO, FeNO = Fractional exhaled nitric oxide.

pulmonary function unit. In our sample, FeNO did not correlate with other respiratory function parameters such as DLCO and FVC. Its elevation in those patients with sequelae, who were the most severely ill during admission, wich could indicate its relationship with the severity of the condition as seen in other studies already discussed[33].

This research has some limitations, it is a single-center study, no pre-infection CT scan was available in all patients included in the study. Previous radiological imaging, either CT or chest X-ray, was available at least 2 years before in 31.94% of the patients. The rest had no compatible respiratory symptoms. Furthermore, it should be borne in mind that pulmonary fibrosis is a condition with a low incidence of 4.6–7.4 per 100,000 inhabitants. However, it also has important strengths: this study has the largest sample to date evaluating FeNO as a biomarker in post-COVID patients and its relationship with possible sequelae at the pulmonary level, presenting clear and statistically robust results. Few blood markers were added in our study, this would be a

possible avenue for future study. These blood markers could be added to FeNO to achieve more complete results in terms of possible screening for fibrotic sequelae.

Conclusion

In conclusion, our study shows an increase in FeNO in those patients who, after admission for severe SARS-CoV-2 pneumonia, present fibrotic interstitial sequelae at three months of follow-up. Furthermore, it shows that, among the different variables related to the presence of these sequelae, it is one of the most predictive. Although further studies are needed, FeNO measurement could be implemented in the follow-up of patients with severe SARS-CoV-2 pneumonia for decision making regarding the request of a HRCT scan for sequelae assessment.

Data availability

The datasets generated and/or analysed during the current study are not publicly available in order to protect patients' privacy but are available from the corresponding author, Diego Ferrer-Pargada (diego jose.ferrer@scsalud.es) on reasonable request.

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References

1. WHO. Director-General's Opening Remarks at the Media Briefing on COVID-19–11. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-oncovid-19-11-march-2020. Accessed 17 July 2021 (2020).
2. Wagner, C. et al. Systemic corticosteroids for the treatment of COVID-19. *Cochrane Database Syst. Rev.* **8**(8), CD014963. 10.1002/14651858.CD014963 (update in: *Cochrane Database Syst. Rev.* 11, CD014963, 2022) (2021).
3. Baden, L. R. et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl. J. Med.* **384** (5), 403–416. https://doi.org/10.1056/NEJMoa2035389 (2021).
4. Barbeta, E. et al. Covid clinic critical care group:. SARS-CoV-2-induced acute respiratory distress syndrome: pulmonary mechanics and Gas-Exchange abnormalities. *Ann. Am. Thorac. Soc.* **17** (9), 1164–1168. https://doi.org/10.1513/AnnalsATS.202005-462RL (2020). PMID: 32579033; PMCID: PMC7462332.
5. Ma, Y. et al. Long-Term consequences of COVID-19 at 6 months and above: A systematic review and Meta-Analysis. *Int. J. Environ. Res. Public. Health.* **19** (11), 6865. https://doi.org/10.3390/ijerph19116865 (2022).
6. Mandal, S. et al. Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. *Thorax* **76** (4), 396–398. https://doi.org/10.1136/thoraxjnl-2020-215818 (2021).
7. Hama Amin, B. J. et al. Post COVID-19 pulmonary fibrosis; a meta-analysis study. *Ann. Med. Surg. (Lond).* **77**, 103590. https://doi.org/10.1016/j.amsu.2022.103590 (2022).
8. Singh, S. J. et al. Respiratory sequelae of COVID-19: pulmonary and extrapulmonary origins, and approaches to clinical care and rehabilitation. *Lancet Respir Med.* **11** (8), 709–725. https://doi.org/10.1016/S2213-2600(23)00159-5 (2023).
9. Alilou, S. et al. Radiological findings as predictors of COVID-19 lung sequelae: A systematic review and Meta-analysis. *Acad. Radiol.* **30** (12), 3076–3085. https://doi.org/10.1016/j.acra.2023.06.002 (2023).
10. Bocchino, M., Rea, G., Capitelli, L., Lieto, R. & Bruzzese, D. Chest CT lung abnormalities 1 year after COVID-19: A systematic review and Meta-Analysis. *Radiology* **308** (1), e230535. https://doi.org/10.1148/radiol.230535 (2023).
11. Sibila, O. et al. Documento de consenso de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR) para el seguimiento clínico post-COVID-19 [Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) Consensus for post-COVID-19 Clinical Follow-up]. *Open Respir. Arch.* **2**(4), 278–283 (2020). https://doi.org/10.1016/j.opresp.2020.09.002 (Spanish).
12. Torres-Castro, R. et al. Respiratory function in patients' post-infection by COVID-19: a systematic review and meta-analysis. *Pulmonology* **27** (4), 328–337. https://doi.org/10.1016/j.pulmoe.2020.10.013 (2021).
13. Pan, X. et al. Increased Circulating levels of SP-D and IL-10 are associated with the development of disease severity and pulmonary fibrosis in patients with COVID-19. *Front. Immunol.* **16**, 1553283. https://doi.org/10.3389/fimmu.2025.1553283 (2025). PMID: 40160824; PMCID: PMC11949947.
14. Safont, B. et al. Lung function, radiological findings and biomarkers of fibrogenesis in a cohort of COVID-19 patients six months after hospital discharge. *Arch. Bronconeumol.* **58** (2), 142–149. https://doi.org/10.1016/j.arbres.2021.08.014 (2022).
15. Korevaar, D. A. et al. Effectiveness of FeNO-guided treatment in adult asthma patients: A systematic review and meta-analysis. *Clin. Exp. Allergy.* **53** (8), 798–808. https://doi.org/10.1111/cea.14359 (2023). Epub 2023 Jun 9. PMID: 37293870.
16. Pianigiani, T. et al. Exploring the interaction between fractional exhaled nitric oxide and biologic treatment in severe asthma: A systematic review. *Antioxid. (Basel).* **12** (2), 400. https://doi.org/10.3390/antiox12020400 (2023). PMID: 36829959; PMCID: PMC9952501.
17. Murugesan, N., Saxena, D., Dileep, A., Adrish, M. & Hanania, N. A. Update on the role of FeNO in asthma management. *Diagnostics (Basel).* **13** (8), 1428. https://doi.org/10.3390/diagnostics13081428 (2023). PMID: 37189529; PMCID: PMC10137365.
18. Cameli, P. et al. Extended exhaled nitric oxide analysis in interstitial lung diseases: A systematic review. *Int. J. Mol. Sci.* **21** (17), 6187. https://doi.org/10.3390/ijms21176187 (2020). PMID: 32867116; PMCID: PMC7503828.
19. Cameli, P. et al. Alveolar nitric oxide as a biomarker of COVID-19 lung sequelae: A pivotal study. *Antioxid. (Basel).* **10** (9), 1350. https://doi.org/10.3390/antiox10091350 (2021).
20. Maniscalco, M. et al. Can FeNO be a biomarker in the post-COVID-19 patients monitoring? *Respir Med.* **193**, 106745. https://doi.org/10.1016/j.rmed.2022.106745 (2022).
21. Betancor, D., Olaguibel, J. M. & Sastre, J. The discrepant role of fractional exhaled nitric oxide in SARS-CoV-2 infection. *J. Investig Allergol. Clin. Immunol.* **32** (5), 417–418. https://doi.org/10.18176/jiaci.0842 (2022).
22. Michel, T. & Feron, O. Nitric oxide synthases: which, where, how, and why? *J. Clin. Invest.* **100** (9), 2146–2152. https://doi.org/10.1172/JCI119750 (1997).
23. Krauss, E. et al. Exhalative breath markers do not offer for diagnosis of interstitial lung diseases: data from the European IPF registry (eurIPFreg) and biobank. *J. Clin. Med.* **8** (5), 643. https://doi.org/10.3390/jcm8050643 (2019).
24. Cameli, P. et al. Evaluation of multiple-flows exhaled nitric oxide in idiopathic and non-idiopathic interstitial lung disease. *J. Breath. Res.* **13** (2), 026008. https://doi.org/10.1088/1752-7163/ab0233 (2019).
25. Ojo, A.S., Balogun, S.A., Williams, O.T. & Ojo, O.S. Pulmonary fibrosis in COVID-19 survivors: predictive factors and risk reduction strategies. *Pulm. Med.* **2020**, 6175964. https://doi.org/10.1155/2020/6175964 (2020).
26. Hua-Huy, T. et al. Distal lung inflammation assessed by alveolar concentration of nitric oxide is an individualised biomarker of severe COVID-19 pneumonia. *J. Pers. Med.* **12** (10), 1631. https://doi.org/10.3390/jpm12101631 (2022).

27. Francistiová, L. et al. Cellular and molecular effects of SARS-CoV-2 linking lung infection to the brain. *Front. Immunol.* **12**, 730088. https://doi.org/10.3389/fimmu.2021.730088 (2021).
28. Armiñanzas, C. et al. Usefulness of the COVID-GRAM and CURB-65 scores for predicting severity in patients with COVID-19. *Int. J. Infect. Dis.* **108**, 282–288. https://doi.org/10.1016/j.ijid.2021.05.048 (2021).
29. Babar, M., Jamil, H., Mehta, N., Moutwakil, A. & Duong, T. Q. Short- and Long-Term Chest-CT findings after recovery from COVID-19: A systematic review and Meta-Analysis. *Diagnostics* **14** (6), 621. https://doi.org/10.3390/diagnostics14060621 (2024).
30. Culver, B. H. et al. Recommendations for a standardized pulmonary function report. An official American thoracic society technical statement. *Am. J. Respir Crit. Care Med.* **196** (11), 1463–1472. https://doi.org/10.1164/rccm.201710-1981ST (2017).
31. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am. J. Respir Crit. Care Med.* **171** (8), 912–930. https://doi.org/10.1164/rccm.200406-710ST (2005).
32. Dweik, R. A. et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am. J. Respir Crit. Care Med.* **184** (5), 602–615. https://doi.org/10.1164/rccm.9120-11ST (2011).
33. Lior, Y. et al. Fractional exhaled nitric oxide (FeNO) level as a predictor of COVID-19 disease severity. *Nitric Oxide.* **124**, 68–73. https://doi.org/10.1016/j.niox.2022.05.002 (2022).
34. Cameli, P. et al. Alveolar concentration of nitric oxide as a prognostic biomarker in idiopathic pulmonary fibrosis. *Nitric Oxide.* **89**, 41–45. https://doi.org/10.1016/j.niox.2019.05.001 (2019). Epub 2019 May 2. PMID: 31054949.
35. Guillen-Del Castillo, A. et al. Prognostic role of exhaled breath condensate pH and fraction exhaled nitric oxide in systemic sclerosis related interstitial lung disease. *Arch. Bronconeumol.* **53** (3), 120–127. https://doi.org/10.1016/j.arbres.2016.09.014 (2017). English, Spanish.
36. Cameli, P. et al. Exhaled nitric oxide in interstitial lung diseases. *Respir Physiol. Neurobiol.* **197**, 46–52. https://doi.org/10.1016/j.resp.2014.03.011 (2014). Epub 2014 Apr 2. PMID: 24703971.

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Author contributions

Guarantor of the paper: DFP Conceptualization: DFP, CAA, JMC. Data curation: DFP, STM, BAB, PAL, JA, FA, CA. Formal analysis: DFP, CAA, MS, CA. Project administration: DFP CAA. Methodology: DFP, CAA, JGOV, JMC. Resources: DFP, JA, PAL. Visualization: CAA BAL, ARG. Supervision: JMC, JGOV, MS. Software: MS, FA. Writing—original draft: DFP, CAA, BAB, STM, CA. Writing—review and editing: DFP, CAA, MS, JGOV, JMC.

Declarations

Competing interests

The authors declare no competing interests.

Ethical declarations

This study complies with internationally accepted standards for research practice and reporting. The Ethics Committee of our Institution approved the study (2018.276). All patients gave informed written consent to take part in this study.

Additional information

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