Evaluation of fractional exhaled nitric oxide during SARS-CoV-2 infection

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The fraction of exhaled nitric oxide (FENO) is a non-invasive marker of T2 inflammation. Measurement of FENO is increasingly used in diagnosis and follow-up of some allergic diseases like asthma. High levels of FENO are correlated with asthma attacks and are being evaluated in other respiratory diseases. Respiratory infections also have a significant effect on FeNO through of an unknown mechanism [1-3]. The increase or decrease of FENO value during clinical viral infection is unclear and seem to be viral microorganism-dependent [4]. The unknown and variable clinical evolution of SARS-CoV-2 infection from asymptomatic to death has force us to investigate new biomarkers that could predict the natural evolution of the disease.

Aiming to assess the potential role of FENO as a marker of severity in cases of COVID-19, we included consecutive subjects over 18 years of age who received care in the emergency department (ED) at Fundación Jimenez Diaz Hospital in Madrid, Spain, with SARS-CoV-2 infection, from January to June 2021. All patients had a positive reverse transcriptase-polymerase chain reaction (RT-PCR) RT-PCR result and/or positive antigen test. A control group was included, consisting of patients presenting respiratory symptoms but with a negative SARS-CoV-2 RT-PCR. The hospital ethics committee approved this study. All patients provided signed informed consent to be included.

FENO was measured in duplicate using the Evernoa device (Eversens, Pamplona, Spain) [5]. The first measurement was taken at baseline, before any therapeutic

intervention in the ED; the second was performed at least 10 days later (infection recovery). Data collected included demographic, clinical, and disease characteristics, presence of atopy (considered as patient´s referred atopy) and other comorbidities presence of pneumonia, treatment, hospital and intensive care unit (ICU) admission if required, blood analysis, and findings on chest x-ray. If the patient was admitted, FENO was measured every 48-72 hours until discharge. The control group included 18 patients.

Quantitative variables were described using mean values and standard deviation (±), and qualitative variables by absolute and relative frequencies. Inter-group comparisons were performed using chi-square test or Fisher's exact test for qualitative variables and ANOVA or Kruskal-Wallis for quantitative variables.P values <0.05 were considered significant

Eighty-two patients were included and divided into 3 groups depending on WHO severity classification [6]: 25 patients with mild infection (uncomplicated upper respiratory tract viral infection), 26 with moderate pneumonia (no initial need for supplemental oxygen), and 31 with severe pneumonia (SpO2 ≤ 93% on room air at baseline). Patient characteristics and laboratory findings are summarized in Table 1 and Figure S1 in supplementary material.

Overall, mean FENO for the sutdy population was in the normal range during infection with no significant variation during the recovery phase (Table 1). There were no significant differences in FENO values according to disease severity or atopy history ($p>0.05$, both). The same applies for control group, no significant difference in FENO values were obtaines between respiratory symptomatic period and recovery.

Repeated FENO was performed only in 29 subjects during hospitalization, as the rest were lost to follow-up or having severe clinical condition. In these patients, a

significant decrease in FeNO was observed (10.9±9.1 ppb at admission vs 2.7±2.8 ppb at last measurement during hospitalization, p=0.03) (Figure S2 supplementary). All these patients were treated with systemic corticosteroids. The initial decrease is likely due to effect of systemic corticosteroids on inducible-NO synthase, and is not explained by the natural evolution of the infection.

Fifteen patients were admitted to the ICU (30% of all hospital-admitted patients). FENO values increased slightly during recovery compared to baseline (15.8±14.2 ppb vs 11.1±1.2 ppb ppb, p=0.12). One patient died, whose initial FENO was 28 ppb, though the cause of the death was non-respiratory complications.

How respiratory infections affect FENO levels is controversial. Kharitonov et al. [1] reported that FENO is increased threefold during clinical upper respiratory tract infections. However, a viral confirmation test was not done in this study. Malka et al. [3] demonstrated a FENO increase in children with acute viral asthma exacerbation with a positive viral nasopharyngeal PCR. Nevertheless, the disease-causing virus was not described. In contrast, Wang et al.[4] showed variable FENO levels in patients with lower respiratory tract infection depending on the isolated virus. Significantly lower values were observed in adenovirus, influenza type A, parainfluenza, rhinovirus, metapneumovirus and respiratory syncytial virus (RSV), on the contrary, bocavirus infection resulted in significantly higher FENO values. Gadish et al.[7] demonstrated that FENO levels were significantly lower during RVS acute infection despite increased FENO production and activity in vitro and animal studies. Salem et al. [8] showed lower FENO levels in recovered patients with previous SARS-CoV-2 pneumonia.

Epithelial inducible nitric oxide synthase (eNOS) expression is increased in some viral infections (e.g., HRV, RSV, Influenza A) [1,2] as well as coronavirus in response to proinflammatory cytokines. Hosts that are deficient in airway NO may have impaired respiratory antiviral defense. Recently, Martel et al. [9] showed increased susceptibility to SARS-CoV-2 infection in patients with low airway NO levels. NO gas therapy has also been investigated as a treatment in patients with severe hypoxemia in SARS-CoV-2 infection.

This is the first study that evaluates FENO levels during acute symptomatic SARS-CoV-2 infection to the best of our knowledge. Study limitations include that the possible respiratory infection was not well defined in the control group and that other respiratory diseases may be a confounder factor and that the atopy status was based on patient's information than on allergy diagnosis confirmation.

In summary, we found FENO levels within the normal range during acute symptoms of SARS-CoV-2 infection, with some increase during the recovery phase, independently of disease severity or the patient's history of atopy. However, in patients treated with steroids, a significant FENO decrease during clinical evolution was more pronounced. These results suggest that FENO is not a good biomarker for diagnosis, assessing severity or prognosis of SARS-CoV-2 infection, although more studies are necessary to confirm this hypothesis.

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Conflict of interests

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Table 1. Demographic and disease characteristics among the patient population. Quantitative variables are expressed as means (standard deviation, SD); qualitative variables are presented as total number of events (percentage). N.S = Not Statistically Significant

	Mild SARS-CoV-2 infection	SARS-CoV-2 pneumonia	SARS-CoV-2 severe pneumonia	Control group (negative SARS-CoV-2)	P value
No. subjects	25	26	31	18	
Demographic characteristics					
Male sex	14 (56%)	21 (80.7%)	18 (58.1%)	10 (52.6%)	NS
Age in years	52.4 ± 15.1	51 ± 14.3	52.3 ±14.7	51.5 ± 14.8	NS
BMI	26.9 ± 5.2	26.9± 5.2	27.2± 5.4	26.5 ± 4.9	NS
Race, Caucasian	16 (64%)	19 (73.1%)	18 (58.1%)	12 (63.2%)	NS
Atopy	7 (28%)	4 (15.4%)	9 (29.0%)	5 (26.3%)	NS
Allergic rhinitis	5 (20%)	4 (15.4%)	6 (19.4%)	4 (21.1%)	NS
Asthma	2 (8%)	3 (11.5%)	4 (12.9%)	4 (21.1%)	NS
No history of smoking	14 (56%)	11 (42.3%)	15 (48.4%)	12 (63.2%)	NS
Current smoker	1 (4%)	5 (19.2%)	1 (3.2%)	5 (26.3%)	NS
Ex-smoker	9 (36%)	11 (42.3%)	15 (48.4%)	2 (10.5%)	NS
Hypertension	1 (4%)[a]	8 (30.7%)[a]	7 (22.6%)	2 (10.5%)	0.02
Diabetes	2 (8%)	6 (23.1%)	2 (6.4%)	1 (5.3%)	NS
Dyslipidemia	0 (0%)	5 (19.2%)	4 (12.9%)	2 (10.5%)	NS
Clinical characteristics					
Days with symptoms	7.1± 4.5	7.2± 4.4	7.4± 4.6	6.9 ± 4.58	NS
Fever	16 (64%)	22 (84.6%)	24 (77.4%)	6 (31.6%)	NS
Cough	22 (88%)	21 (80.7%)	27 (87.1%)	11 (57.9%)	NS
Breathlessness	7 (28%)	12 (46.1%)	25 (80.6%)	6 (31.6%)	NS
Presence of pneumonia	0 (0%)	26 (100%)	31 (100%)	2 (10.5%)	NS
Bilateral pneumonia	0 (0%)	18 (69.2%)	30 (96.7%)	1 (5.5%)	NS
Hospital admission	2 (8%)[b]	17 (65.4%)[b]	31 (100%)[b]	0 (0%)[b]	<0.01
ICU admission	0 (0%)	2 (7.7%)	13 (41.9%)	0 (0%)	NS

Death	0 (0%)	0 (0%)	1 (3.2%)	0 (0%)	NS
Disease-related complementary tests					
Lymphocytes (linf/µl)	1244.1± 721.7	1229.4± 710.4	1201.7±727.9	1212.5± 853	NS
Eosinophils (eos/µl)	30.2 ± 146.4	29.2± 146.4	29.5±156.7	41.1± 173.3	NS
FeNO at baseline (ppb)	13.0± 12.4	12.9± 12.2	12.9± 12,9	13.2 ± 12.4	NS
FeNO control at recovery phase (ppb)	21.6± 23.2	20.6± 22.9	21.3± 24,9	21.5 ± 23.2	NS
FeNO difference between the two measurements (ppb)	+ 8.6 (p= 0.13)	+ 7.6 (p=0.2)	+ 8.36 (p=0.4)	+ 8.35 (p=0.6)	NS

[a]Significant difference was obtained in presence of hypertension between mild and moderate SARS-CoV-2 infection ($p<0.05$)

[b]Significant difference was obtained in hospital admission in all inter-group comparison (all $p<0.01$) except between mild infection and control group ($p=0.5$)