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More questions than answers for the use of inhaled nitric oxide in COVID-19

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ABSTRACT

Inhaled nitric oxide (iNO) is a potent vasodilator approved for use in term and near-term neonates, but with broad off-label use in settings including acute respiratory distress syndrome (ARDS). As an inhaled therapy, iNO reaches well ventilated portions of the lung and selectively vasodilates the pulmonary vascular bed, with little systemic effect due to its rapid inactivation in the bloodstream. iNO is well documented to improve oxygenation in a variety of pathological conditions, but in ARDS, these transient improvements in oxygenation have not translated into meaningful clinical outcomes. In coronavirus disease 2019 (COVID-19) related ARDS, iNO has been proposed as a potential treatment due to a variety of mechanisms, including its vasodilatory effect, antiviral properties, as well as anti-thrombotic and anti-inflammatory actions. Presently however, no randomized controlled data are available evaluating iNO in COVID-19, and published data are largely derived from retrospective and cohort studies. It is therefore important to interpret these limited findings with caution, as many questions remain around factors such as patient selection, optimal dosing, timing of administration, duration of administration, and delivery method. Each of these factors may influence whether iNO is indeed an efficacious therapy - or not - in this context. As such, until randomized controlled trial data are available, use of iNO in the treatment of patients with COVID-19 related ARDS should be considered on an individual basis with sound clinical judgement from the attending physician.

1. Introduction

Inhaled nitric oxide (iNO) is a potent vasodilator approved by the U. S. Food and Drug Administration to improve oxygenation and reduce the need for extracorporeal membrane oxygenation in term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension (PH) in conjunction with ventilatory support and other appropriate agents [1]. When inhaled, NO selectively vasodilates the pulmonary vascular bed. iNO acts by stimulating soluble guanylate cyclase, thereby increasing the production of cyclic guanosine monophosphate (cGMP). In turn, cGMP activates protein kinase G (PKG) before being degraded by phosphodiesterase 5. In smooth muscle tissue, elevations in PKG in turn modulates the activity of several ion channels, including calcium-activated potassium channels and sodium/calcium exchangers, leading to relaxation of the smooth muscle and cell hyperpolarization [2]. Once NO diffuses into the bloodstream, it is rapidly scavenged, binding to hemoglobin and other proteins and compounds to form NO derivatives including S-nitrosothiols, nitrosylhemoglobin, and other soluble carriers of NO in the bloodstream, which can be reduced back to NO under distinct physiological conditions [3–9]. Consequently, due to the rapid scavenging of NO in the bloodstream, systemic hemodynamics are largely unaffected with little risk of systemic hypotension and iNO is thus considered to be highly selective to the pulmonary vasculature. In general, iNO is considered to have a favorable risk-benefit profile, leading to its approval by the US Food and Drug Administration in 1999 [1]. However, safety considerations such as monitoring for elevations in methemoglobin, prevention of abrupt discontinuation or interruption of iNO, which can result in rebound pulmonary hypertension, and monitoring for nitrogen dioxide levels, are important for safe application of iNO [1]. As an inhaled therapy, iNO is distributed to well-ventilated portions of the lung and improves oxygenation by matching alveolar ventilation and perfusion.

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2. Summary of iNO use in ARDS and previous SARS-CoV-1 outbreak

In acute respiratory distress syndrome (ARDS), off-label use of iNO has been shown to decrease pulmonary capillary pressure and pulmonary transvascular albumin flux, selectively vasodilate the pulmonary vasculature, and improve oxygenation [10]. However, despite transient improvements in oxygenation, these physiological effects have not translated into meaningful clinical outcomes, with multiple randomized controlled trials (RCTs) demonstrating no benefit on the duration of ventilatory support or mortality in ARDS [11]. In a small study of patients with Severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) infection conducted in China, iNO reversed PH, improved oxygenation, and reduced the duration of ventilatory support [12]. Aside from improving oxygenation, iNO may also be of benefit due to its antiviral activity [13], including inhibition of SARS-CoV-1 viral replication [14] and protection of cells *in vitro* from SARS-CoV-1 infection [15], as well as its anti-thrombotic/-inflammatory effects [16,17].

3. Rationale for use of iNO in COVID-19

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, resulting in coronavirus 2019 disease (COVID-19), can result in respiratory failure and death. Severe patients frequently present with hypoxemia and are at high risk of developing COVID-19-associated ARDS, requiring intensive care including invasive mechanical ventilation (IMV) and pharmacotherapy. As a potent and selective pulmonary vasodilator, iNO has been appraised as an attractive adjunctive therapy that may be beneficial to COVID-19 patients with or without ARDS [13,16,18–25]. Additionally, the aforementioned antiviral, anti-thrombotic, and anti-inflammatory effects of iNO have putative benefit in the context of COVID-19.

Despite the physiological rationale for iNO use in COVID-19, current *clinical* guidelines do not recommend its routine use for this condition with exception to consider iNO as a rescue/adjunctive therapy and to discontinue iNO if no quick response is observed [26]. To date, no prospective RCTs studying the efficacy and safety of iNO in COVID-19 have been published. Numerous case series, cohort studies, and retrospective investigations have been published, however, with conflicting results [27–47] (Table 1). A recent systematic review of studies on iNO in COVID-19 [48] found that similar to findings in non-COVID-19 ARDS, iNO improves oxygenation in COVID-19-associated ARDS while having no apparent effect on mortality.

Several factors must be considered when interpreting the conclusions of this systematic review, however. First, the majority of included studies were retrospective, which introduces the possibility that other confounders influencing mortality were not accounted for in these patients [49]. Second, the sample sizes were small, and none enrolled more than 50 patients, with the exception of one which was designed to study almintrine infusion and also enrolled some patients who received iNO [47]. Third, many were case series with no control or comparator group. Fourth, studies varied on the severity of hypoxemia in patients enrolled, the timing of when iNO was initiated, the dose of iNO given, and the duration that patients stayed on iNO. A recent cohort study found ARDS severity influenced the association of iNO and mortality in pediatric patients, with those with greater severity hypoxemia benefitting more from iNO [50]. Together, these factors perhaps raise more questions than they answer (Fig. 1), and in the absence of high quality RCT data, it is unclear if iNO is beneficial in COVID-19.

4. Key unanswered questions regarding use of iNO in COVID-19

If iNO is indeed beneficial in COVID-19, the first question that must be answered is which patients, if any, are the best candidates for treatment? Is it the mild patient who received prophylactic iNO to prevent disease progression and hypoxemia or the severe patient with refractory hypoxemia who requires rescue treatment? While these situations are not mutually exclusive, the rationale for use is understandably different in these clinical situations.

Finding clinical indicators or biomarkers that may predict patients who are more likely to respond to iNO treatment would be a useful tool to aid clinicians in deciding whether or not to initiate iNO. For example, PH in COVID-19 patients may be predictive of responsiveness given the established pulmonary vasodilating effect of iNO. Biomarkers such as circulating IL-6 levels may be informative as to whether patients have excessive inflammation, and although at present no data are available correlating IL-6 levels with iNO responsiveness, IL-6 and other biomarkers may nevertheless be informative. For example, in a retrospective cross-sectional study by Herranz et al. [35], patients who received iNO tended to have IL-6 levels three times higher than patients who did not receive iNO. However, no data were presented on whether IL-6 levels influenced iNO responsiveness.

In non-COVID-19 ARDS, distinct subphenotypes (hyperinflammatory and hypoinflammatory) have been identified using sophisticated unbiased methods such as latent class analysis and machine learning algorithms [51,52]. These methods were applied in post-hoc secondary analyses of previously completed RCTs studying statins in ARDS and could similarly by applied in the context of iNO for COVID-19 related ARDS [53,54]. In a secondary analysis of the SAILS trial studying rosuvastatin therapy in ARDS, the authors did not find differential responses based on ARDS subphenotype (hyper-vs hypo-inflammatory) [54], and the overall trial did not find a clinical benefit of rosuvastatin therapy in ARDS [55]. In contrast, the secondary analysis of the HARP-2 trial studying simvastatin therapy in ARDS did find differential responses with the hyperinflammatory subphenotype benefitting from therapy whereas the hypoinflammatory subphenotype did not [53]. The overall HARP-2 trial findings did not show a clinical benefit from simvastatin therapy in ARDS [56].

While these secondary analyses have limitations since they were not prospectively defined and applied retrospectively to previously collected data, a more recent single center observational investigation conducted early in the COVID-19 pandemic revealed that the hyperinflammatory subphenotype of COVID-19-associated ARDS had improved mortality with corticosteroid treatment whereas the hypoinflammatory subphenotype had worse mortality with corticosteroid treatment [57]. Together, these data highlight the need for better predictive indicators of which ARDS patients (including those with COVID-19-associated ARDS) are more likely to respond to specific treatments. In fact, a recent Position Paper reiterated the need to advance a precision medicine approach to ARDS to better account for the clinical and biological heterogeneity that modifies treatment responsiveness in ARDS [58].

Next, the dose and timing of when iNO should be given are other important considerations. Again, should iNO be given early in the disease course to prevent disease progression, or would it be more beneficial in later-stage disease as a rescue therapy? In neonates with hypoxic respiratory failure, one RCT showed that earlier administration at an oxygenation index (OI) of 15–25 vs. >25 improved oxygenation, but did not reduce the incidence of the composite outcome of extracorporeal membrane oxygenation or mortality [59]. A separate trial of early iNO administration in neonates (given at an OI of 10–30) found that earlier initiation of iNO improved oxygenation as well and also reduced the probability of developing severe hypoxemic respiratory failure, defined as an OI > 40 [60]. Acknowledging that these data are from neonates, a distinct population from the majority of patients with severe COVID-19 infection, these data may still be informative in demonstrating that earlier use of iNO to improve oxygenation may be a useful application to slow or prevent disease progression in the setting of COVID-19.

Along with timing of administration, another key question is what dose should be given? The on-label recommended dose for neonates is 20 ppm [1], and an RCT in neonates with hypoxic respiratory failure demonstrated that dose increases above 20 ppm up to 80 ppm did not

Table 1
Summary of Studies on iNO in COVID-19.

Citation	Study Design	Population	Enrollment	Dosing	Key Findings
Abou-Arab et al., 2020 [27]	Single-center prospective observational study	Adults admitted to ICU for COVID-19 severe pneumonia per WHO case definition	N = 34	10 ppm iNO	– 22 of 34 patients (65%) were "responders", defined as an increase in P_aO_2/F_iO_2 over 20% over 30 min following iNO administration – PEEP, RS compliance, and driving pressure remained unchanged – P_aO_2/F_iO_2 was significantly lower at baseline in the responders group compared to the non-responders group (70 [63–100] vs 134 [83–173] mmHg, respectively, $P < 0.0001$) but was similar between groups after iNO administration (144 [107–175] vs 125 [92–144] mmHg, respectively, $P = 0.068$)
Bagate et al., 2020 [28]	Single-center prospective	Intubated adult COVID-19 patients with persistent severe hypoxemia (P_aO_2/F_iO_2 <150 mmHg)	N = 10	10 ppm iNO for 30 min followed by iNO+10 μg/kg/min of almitrine for 30 min in the supine position after 16–18 h of proning	– P_aO_2/F_iO_2 increased from median 102 (IQR 89–134) mmHg at baseline to 124 (108–146) mmHg after iNO ($P = 0.13$) to 180 (132–206) mmHg after iNO and almitrine ($P < 0.01$) – Responders defined as P_aO_2/F_iO_2 increase ≥20% or 20 mmHg – P_aO_2 increased by >50% in 7 of 10 patients with iNO-almitrine combination; 1 non-responder had an intra-cardiac shunt related to patent foramen ovale
Cardinale et al., 2020 [29]	Single-center, retrospective observational study	COVID-19 patients with ARDS and P_aO_2/F_iO_2 <120 mmHg	N = 20 (N = 10 receive iNO along; N = 13 received almitrine alone; N = 7 received both iNO + almitrine)	10–20 ppm iNO and/or 0.5 mg/kg almitrine, at the discretion of the attending physician	– Responders defined as P_aO_2/F_iO_2 increase ≥20% – With iNO alone, median increase in P_aO_2/F_iO_2 was 2.2% (95% CI 1.3–12) from 88 (range 73–110) to 94 (74–116) mmHg; no significant difference between patients who received 10 ppm vs 20 ppm; no patient was a responder – With almitrine alone, median increase in P_aO_2/F_iO_2 was 1.9% (95% CI -4.8-11) from 101.2 (range 69.1–120) to 108 (64.5–147) mmHg; only one patient was a responder – With both iNO + almitrine, median increase in P_aO_2/F_iO_2 was 5% (95% CI 1.4–7.8) from 95 (range 73–110) to 102 (74–116) mmHg; no patients were responders
Chandel et al., 2021 [30]	Multicenter retrospective observational cohort study	Adult COVID-19 patients treated with HFNC (excluding patients who were previously intubated and placed on HFNC as a weaning modality)	N = 272, control group N = 206, iNO group N = 66; N = 11 in iNO group had incomplete documentation and removed from final analysis, leaving N = 55 in final analysis for iNO group	20 ppm iNO, with option to increase to 40 ppm if oxygen saturation did not increase by ≥ 5% after 1 h of therapy; if no response after 1 h at 40 ppm, discontinuation of iNO recommended; responders to iNO weaned to lowest effective dose	– Responders defined as improvement in supplemental oxygen requirements – After 12 h of continuous iNO support, 26 of 55 patients (47.3%) had an improvement in supplemental oxygen requirements and 29 of 55 (52.7%) had unchanged or increased supplemental oxygen requirements – Patients who received iNO had lower rates of AKI (control 69 (33.5%) vs iNO group 13 (19.7%), $P = 0.044$) and longer hospital length of stay (control 13 [10–19.5] days vs iNO group 17.5 [12–32] days, $P < 0.001$)

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Table 1 (*continued*)

Citation	Study Design	Population	Enrollment	Dosing	Key Findings
					– No difference in death (P = 0.855) or need for ECMO (P = 0.369)
DeGrado et al., 2020 [31]	Single center, retrospective observational study	Adult patients with COVID-19 and ARDS admitted to any ICU who receive iNO or iEPO while mechanically ventilated	N = 38; N = 11 received iNO after initially receiving iEPO	iEPO given as first-line pulmonary vasodilator at 0.01–0.05 mcg/kg/min; transitioned to iNO at 1–80 ppm if <10% improvement in P_aO_2/F_iO_2; iNO initiated at 20 ppm with recommendation to titrate up to 80 ppm if P_aO_2 does not increase ≥10%	– Median change in P_aO_2/F_iO_2 was 16.7% (IQR 1.6–25.8%) in iNO group – 7 of 11 iNO patients (63.4%) had a P_aO_2/F_iO_2 response ≥10%
Feng et al., 2021 [32]	Single center retrospective case series	Critically ill adult COVID-19 patients with elevated PASP and acute respiratory failure or shock requiring mechanical ventilation	N = 5 (N = 3 received iNO)	10–20 ppm iNO	– 2 of 3 patients (66.7%) had PASP return to normal after iNO – All 3 iNO patients had improvements in P_aO_2/F_iO_2 ○ Case 1 from 88 to 124 mmHg ○ Case 2 from 51 to 118 mmHg ○ Case 3 from 146 to 244 mmHg
Ferrari et al., 2020 [33]	Single center case series	Adult COVID-19 patients receiving invasive mechanical ventilation with P_aO_2/F_iO_2 around or below 100 mmHg	N = 10	20 ppm iNO for 30 min	– No change in P_aO_2/F_iO_2 following iNO (81 ± 19 to 84 ± 22 mmHg, P = 0.325)
Garfield et al., 2021 [34]	Retrospective observational study	Adult COVID-19 patients admitted to the ICU with at least moderate ARDS (P_aO_2/F_iO_2 <26.7 mmHg/ 3.56 kPa)	N = 35	20 ppm iNO; one patient treated at 40 ppm iNO	– P_aO_2/FiO_2 increased significantly within 24 h of iNO initiation (13.6 [3.9] vs 17.4 [5.5] kPa, P < 0.001) – OI significantly reduced following iNO (20.6 [15.2–24.0] vs 14.4 [11.9–20.8], P < 0.001) – 23 of 35 patients (65.7%) patients responded to iNO at 24 h per pre-defined criteria of P_aO_2/F_iO_2 ≥1.33 kPa – Responder had significantly lower baseline P_aO_2/F_iO_2 ratio (12.1 [2.8] vs 16.3 [4.4], P < 0.01) and higher baseline OI (21.6 [6.3] vs 16.1 [5.2], P < 0.01) than non-responders
Herranz et al., 2021 [35]	Single-center retrospective cross-sectional study	Adults admitted to the ICU with severe COVID-19 undergoing mechanical ventilation for at least 48 h	N = 34 (N = 15 control, N = 12 iNO, N = 7 excluded)	20–30 ppm iNO and increased up to 40 ppm maximal dose, according to P_aO_2 response	– iNO group had longer time under mechanical ventilation, longer hospitalization, and required more time under neuromuscular blockade (statistics not reported) – IL-6 levels tended to be three times higher in iNO group (statistics not reported) – Sustained increase of ≥20% in P_aO_2/F_iO_2 with iNO (statistics not reported) – Mortality similar in both groups (statistics not reported)
Heuts et al., 2020 [36]	Case report	Male COVID-19 patient with severe ARDS on veno-venous ECMO	N = 1	20 ppm iNO, increased to 30 ppm; iNO initiated after iloprost treatment	– P_aO_2 increased from 52 mmHg to 61 mmHg after 1 h, then remained stable (66 mmHg at 12 h; 64 mmHg at 24 h after iNO initiation) – Improved recirculation to 22% after 24 h – Cardiac output improved from 6.0 to 7.5 L/min at 24 h after iNO initiation
Laghlam et al., 2021 [37]	Single-center, observational, open-label study	Adult COVID-19 patients in the ICU with moderate to severe ARDS (P_aO_2/F_iO_2<200 mmHg)	N = 12	10 ppm iNO for 30 min, followed by combination treatment with 10 ppm iNO + 8 μg/kg/min almitrine for 30 min, followed by 30 min of almitrine alone	– No significant change in P_aO_2/F_iO_2 from baseline with iNO (146 ± 48 mmHg vs 185 ± 73 mmHg, P = 0.49) – After combined iNO + almitrine, P_aO_2/F_iO_2 improved significantly from 146 ± 48 mmHg to 255 ± 90 mmHg (P = 0.005)

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Table 1 (*continued*)

Citation	Study Design	Population	Enrollment	Dosing	Key Findings
					– With almitrine alone, P_aO_2/F_iO_2 maintained significantly higher from baseline (146 ± 48 mmHg) to 238 ± 98 mmHg ($P = 0.02$) – Response of ≥20% increase in P_aO_2/F_iO_2 was observed in 50% of patients after iNO alone, in 92% of patients after combination iNO + almitrine, and 75% of patients with almitrine alone
Longobardo et al., 2021 [38]	Single-center, retrospective, observational, case-control study	Adult ARDS patients with COVID-19 compared to historical control cohort of adult ARDS patients without COVID-19	N = 245 (N = 154 COVID-19 patients, of which N = 27 received iNO; N = 91 control patients, of which N = 20 received iNO); N = 7 COVID-19 iNO patients and N = 6 non-COVID iNO patients excluded because they died <24 h from iNO initiation	10–20 ppm iNO, titrated to maximal effect over at least 24 h	– Change in P_aO_2/F_iO_2 was smaller in COVID-19 ARDS patients who received iNO (3% [IQR 17–26%]) compared to non-COVID-19 ARDS patients who received iNO (47% [IQR 6–54%]) ($P = 0.045$) – No difference in rate of response, defined as >10% increase in P_aO_2/F_iO_2 (n = 8 [40%] in COVID-19 ARDS group vs n = 10 [77%] in non-COVID-19 ARDS group, $P = 0.07$) – No difference in PEEP, MAP, tidal volume, driving pressure, compliance, fluid balance, CRP, or days from ICU admission to iNO initiation between COVID-19 ARDS group and non-COVID-19 ARDS (all $P > 0.05$)
Lotz et al., 2021 [39]	Single-center, retrospective observational study	Adult COVID-19 patients with ARDS	N = 7	20 ppm iNO for 15–30 min	– P_aO_2 increased from median 78.2 (IQR 64.5–101.5) to 105 (78.5–144.5) mmHg, $P = 0.0313$ – S_aO_2 unchanged from median 94.8 (IQR 92.2–99.2) to 99.4 (95.4–99.8) %, $P = 0.0754$ – No change mPAP, PCWP, or PVR (all $P > 0.05$)
Lubinsky et al., 2022 [40]	Multi-center, retrospective observational cohort study	Adult patients with COVID-19 receiving invasive mechanical ventilation	N = 84 (N = 69 received iNO, N = 15 received iEPO)	10–40 ppm iNO, determined by the treating clinician; or iEPO at 50 ng/kg/min based on IBW and titrated by the treating intensivist as tolerated based on clinical response	– No significant change in P_aO_2/F_iO_2 after initiation of iNO (mean difference −4.1 mmHg, 95% CI -17.3-9.0, $P = 0.54$) or iEPO (mean difference −3.4 mmHg, 95% CI -19.7-12.9, $P = 0.66$) – No significant change in OI after initiation of iNO (mean difference 2.1, 95% CI -0.04-4.2, $P = 0.054$) or iEPO (mean difference −3.4, 95% CI -19.7-12.9, $P = 0.75$)
Parikh et al., 2020 [41]	Single-center observational study	Adult, non-intubated COVID-19 patients	N = 39	30 ppm iNO	– 21 or 39 (53.9%) patients did not require invasive mechanical ventilation after iNO treatment – SF ratio (S_pO_2/F_iO_2, surrogate for P_aO_2/F_iO_2 ratio) improved in non-intubated patients by 54.9 ($P = 0.0078$) – CRP and ferritin did not significantly change after iNO treatment – D-dimer levels increased in 25 of 39 (64.1%) patients with a median change of 115 ng/mL ($P = 0.0052$)
Robba et al., 2021 [42]	Single-center, prospective observational study	Adult COVID-19 patients with ARDS	N = 22 (N = 9 received iNO)	20 ppm iNO, followed by titration according to patient needs and ABGs	– P_aO_2/F_iO_2 increased from median 65 (IQR 67–73) to 72 (67–73) mmHg, $P = 0.015$
Safaee Fakhr et al., 2020 [43]	Single-center, prospective cohort study	Pregnant patients with severe or critical COVID-19	N = 6	160–200 ppm iNO over 30–60 min twice per day; 2 patients who were intubated remained on <40	– All patients had rapid subjective relief of shortness of breath, decreased respiratory rate, and

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Table 1 (*continued*)

Citation	Study Design	Population	Enrollment	Dosing	Key Findings
				ppm continuous iNO until extubation, at which time high-dose treatments resumed	decreased CRP levels after treatment – In 3 patients who had baseline hypoxia, systemic oxygenation increased – 3 patients delivered a total of four neonates during hospitalization; at 28-day follow-up, all 3 patients and their newborns were in good condition – Remaining 3 patients discharged while remaining pregnant; 2 subsequently delivered without complication and 1 had a late preterm birth at 36 weeks of gestation
Tavazzi et al., 2020 [44]	Single-center observational study	Adult COVID-19 patients undergoing mechanical ventilation with refractory hypoxemia and/or right ventricular dysfunction	N = 72 (N = 16 received iNO)	25 ppm (IQR 20–30) iNO	– Overall, iNO did not improve oxygenation, by P_aO_2/F_iO_2 (median 91.7 [62.1–109.2] vs 91.5 [67.1–106.7], $P = 0.274$) – 4 of 16 patients who received iNO (25%) were responders (defined as >20% increase in P_aO_2/F_iO_2), with a median increase in P_aO_2/F_iO_2 of 26.9% (IQR 24.1–45.5)
Wiegand et al., 2020 [45]	Single-center, retrospective observational study	Adult COVID-19 patients who were spontaneously breathing with clinically deteriorating respiratory conditions despite best practice	N = 5	160 ppm iNO for 30 min twice per day	– S_pO_2/F_iO_2 remained stable during and after iNO irrespective of hypoxemia status – No changes in mean arterial pressure, heart rate, or respiratory rate during or after iNO
Ziehr et al., 2021 [46]	Single-center, retrospective cohort study	Adult patients with COVID-19 and ARDS treated with mechanical ventilation and prone positioning in the ICU	N = 122 (N = 12 received iNO)	20–80 ppm iNO, in the supine position prior to prone positioning	– 10 of 12 patients (83%) experienced an increase in P_aO_2/F_iO_2 with iNO – P_aO_2/F_iO_2 increased with iNO from median 136 (IQR 77–168) to 170 (138–213), $P = 0.003$ – Median improvement in P_aO_2/F_iO_2 with iNO was 31.6% (19.4–42.6%) – Subsequent prone positioning while receiving iNO increased P_aO_2/F_iO_2 further from 145 (122–183) to 205 (150–232), $P = 0.017$

ABG = arterial blood gases; AKI = acute kidney injury; ARDS = acute respiratory distress syndrome; COVID-19 = coronavirus disease 2019; CRP = C-reactive protein; ECMO = extracorporeal membrane oxygenation; F_iO_2 = fraction of inspired oxygen; HFNC = high flow nasal cannula; ICU = intensive care unit; iEPO = inhaled epoprostenol; iNO = inhaled nitric oxide; MAP = mean airway pressure; mPAP = mean pulmonary artery pressure; OI = oxygenation index; P_aO_2 = partial pressure of oxygen in arterial blood; PASP = pulmonary artery systolic pressure; PCWP = pulmonary capillary wedge pressure; PEEP = positive end-expiratory pressure; PVR = pulmonary vascular resistance; RS compliance = respiratory lung compliance; S_aO_2 = arterial oxyhemoglobin saturation; S_pO_2 = peripheral oxyhemoglobin saturation; WHO = World Health Organization. Note: A study by Caplan et al. [47] which studied almitrine infusion, with most patients also receiving iNO, was excluded from summary in this table because of insufficient information (number of patients who received iNO not reported, dose of iNO given not reported).

provide additional benefit nor did it increase the rate of responders in the study population [61]. Studies in ARDS have used doses ranging from 5 to 80 ppm [11], but clear superiority of any particular dose has not been identified. Similarly, doses have varied widely in reported COVID-19 studies [48]. In non-COVID-19 ARDS, a sensitization to iNO has been observed in patients who received iNO over the course of 96 h [62]. The resulting leftward shift in the "inverted-U shaped" dose-response curve means that if patients continued receiving the same dose they were initially given, there may be a loss of therapeutic effect, and any consequent physiological or clinical benefit. These data suggest then, that perhaps it would be necessary to titrate the dose to given to COVID-19 patients to achieve the optimal therapeutic effect, and that this dose may require continuous monitoring and adjustment until the underlying hypoxemia resolves.

Aside from dose selection, delivery method (IMV, high-flow nasal cannula [HFNC], non-invasive ventilation [NIV], etc.) and duration of treatment are additional factors to consider. In patients with severe as opposed to critical COVID-19, ventilatory support may be required, but patients may not be intubated, and therefore delivery of iNO by NIV, nasal cannula, or HFNC may be a reasonable method for providing therapy without the need for intubation. Ongoing studies are using both continuous and intermittent dosing strategies, and it is unclear whether one strategy is superior to the other. More likely, both strategies may be useful depending on the context of iNO use (goals of treatment, patient selection, dose delivered, etc.). Finally, defining weaning criteria and response criteria are also important. In non-COVID-19 ARDS studies, the duration of therapy varied widely from several hours to up to 30 days. In light of the potential sensitization to iNO and the dosing considerations described above, understanding the optimal duration of therapy is another area that requires further investigation.

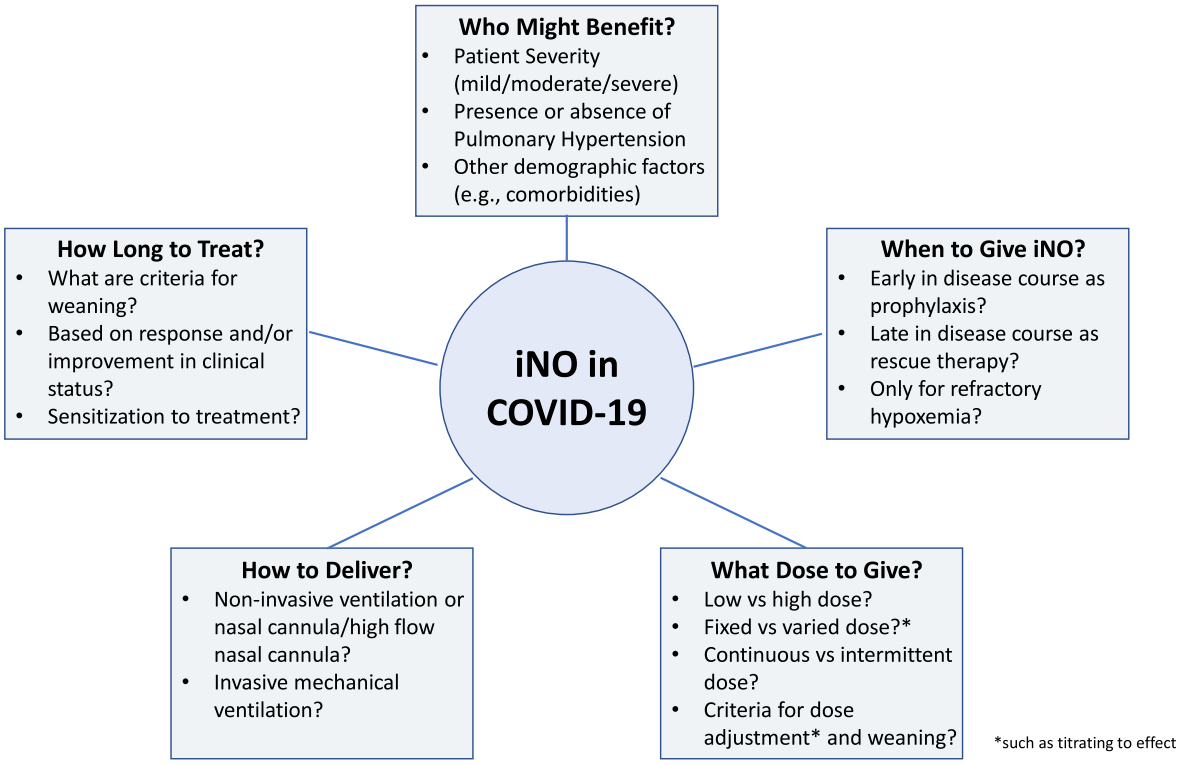

Fig. 1. Key unanswered questions regarding iNO use in COVID-19.

5. Synopsis of registered clinical trials studying iNO in COVID-19 in the United States

Presently, 7 studies on iNO in COVID-19 have been registered in ClinicalTrials.gov in the United States (Table 2), but results from these trials have not been published yet. Of these, NO-COVID-19 (NCT04388683, an open-label proof of concept trial) and COViNOX (NCT04421508, a Phase 3 RCT using the investigational INOpulse® device, which is not yet approved by the FDA) were sponsored or supported by Bellerophon Pulse Technologies and have both been terminated (NO-COVID-19 stopped at Bellerophon's request and COViNOX due to futility). The COViNOX study was terminated after enrolling 191 patients after a pre-specified interim analysis of the first 100 patients randomized [63]. The rate of occurrence of the primary endpoint of respiratory failure or death was lower than anticipated and the independent data monitoring committee recommended placing the study on clinical hold, until it was eventually terminated. Importantly however, no safety concerns were identified in the interim analysis.

Four other studies are sponsored by Massachusetts General Hospital: the NoCovid (NCT04305457), NOSARSCOVID (NCT04306393), NOpreventCOVID (NCT04312243), and NO COV-ED (NCT04338828) studies, which are all presently listed as "Active, not recruiting." Comparing the actual enrollment reported with the estimated enrollment, it appears the NoCovid, NOpreventCOVID and NO COV-ED studies were all stopped early for undisclosed reasons. The NOSARSCOVID study, the largest of the 4, appears to have completed enrollment with actual reported enrollment matching the estimated enrollment.

Similar to the COViNOX study, the NoCovid study enrolled hospitalized COVID-19 patients not requiring ventilation or HFNC, but delivered iNO at a substantially higher concentration (140–180 ppm) for 20–30 min, twice per day for 14 consecutive days. In contrast, the NOSARSCOVID study was a blinded RCT aiming to enroll 200 patients with severe COVID-19 who were intubated and mechanically ventilated. Dosing was set at 80 ppm for 48 h followed by 40 ppm, followed by weaning once patients maintained a PaO_2:F_iO_2 ratio $\geq$300 mmHg for at least 24 h, consecutively. The NO COV-ED study aimed to determine whether iNO improves short term respiratory status, prevents future hospitalization, and improves the clinical course in patients admitted to the emergency department with COVID-19. The NOpreventCOVID study aimed to assess whether intermittent delivery of iNO in air at a high dose may protect healthcare workers from SARS-CoV-2 infection.

A separate trial sponsored by Beyond Air Inc (NCT04397692) is still recruiting patients, although this is a small proof of concept trial which is open-label and aiming to enroll just 20 patients and using the LungFit™ device, an investigational iNO delivery device which is not yet approved by the FDA.

It should be apparent that these studies vary greatly in their dosing protocol, the patient population studied, and the primary outcome measure (Table 2). While some studies aim to assess the effect of iNO on oxygenation, others measure more "real-world" outcome such as mortality and rate of respiratory failure or escalating ventilatory support requirements. Further, patient illness severity has varied greatly between these studies with some excluding those requiring mechanical ventilation, others including only those intubated or requiring mechanical ventilation, and others not specifying inclusion criteria beyond having a diagnosis of COVID-19. The doses have also varied widely between these trials, ranging from 80 to 300 ppm or 125 mcg/kg/IBW/h and do not seem to be dependent on illness severity of the patient studied. Lastly, dose frequency and duration are also inconsistent between trials with some administering a single dose, some a dose twice per day for 14 days, others using clinical end-points such as resolution/discharge to determine dose duration, and only 1 implementing a weaning protocol. While these investigations should provide higher-quality evidence regarding whether iNO is indeed a useful therapeutic in COVID-19, further investigation is still necessary to definitively identify which, if any, populations might benefit from iNO treatment, and what optimal dosing strategies and duration may be.

Table 2
Summary of iNO trials registered on ClinicalTrials.gov.

Trial Name	Study Status	Population	Enrollment (actual/ anticipated)	Blinding	Dosing	Primary Outcome Measure
COViNOX (NCT04421508)	Terminated (futility)	Hospitalized COVID-19 patients: – $S_pO_2 \leq 92\%$ OR on supplemental oxygen (≤10 L/min) – COVID-19 pneumonitis – Not requiring assisted ventilation	191/500 (38%)	Double-blinded	125 mcg/kg IBW/h 24 h/d for ≤14 d or until resolution/ discharge	Mortality or respiratory failure (within 28 d of treatment)
NOSARSCOVID (NCT04306393)	Active (not recruiting)	COVID-19 patients admitted to ICU: – Intubated (≤72 h) – Mechanically ventilated (tidal volume ≥3 cc/kg IBW)	200/200 (100%)	Single-blinded	80 ppm for 48 h followed by 40 ppm and weaning protocol	Change in PaO2 from enrollment to 48 h
NO-COVID-19 (NCT04388683)	Terminated (Collaborator requested)	Hospitalized COVID-19 patients: – Reporting dyspnea – $S_pO_2 \leq 94\%$ OR supplemental oxygen (≤5 L/min) – Not requiring intubation, HFNC, or NIV – ≥ 2 risk factors for clinical worsening (≥60 years, T2DM or pre-diabetes, BMI ≥30 kg/m^2, hypertensive)	10/42 (24%)	Open Label	125 mcg/kg IBW/h for unspecified duration	Prevention of progressive systemic de-oxygenation, with escalation to higher levels of oxygen and ventilatory support or death assessed via 7-point severity scale (within 28 d of treatment)
NO–COV-ED (NCT04338828)	Terminated (absence of patients meeting inclusion criteria)	COVID-19 patients admitted to the ED: – With ≥1 of the following (RR ≥ 24 bpm, new cough, new atypical chest pain, new dyspnea, $S_pO_2 < 97\%$, chest X-ray with new changes) – Cleared for discharge – Requiring supplemental oxygen to maintain $S_pO_2 > 94\%$	47/260 (18%)	Triple-Blinded	140–300 ppm for 20–30 min	Rate of return visits to ED (within 28 d of treatment)
NOpreventCOVID (NCT04312243)	Active (not recruiting)	Healthcare workers scheduled to work with COVID-19 patients ≥ 3 d/wk	24/460 (5%)	Open-Label	160 ppm for 15 min, 2 times daily	COVID-19 diagnosis (within 14 d of treatment)
NoCOVID (NCT04305457)	Active (not recruiting)	Hospitalized COVID-19 patients: – With ≥1 of the following (fever, RR ≥ 24 bpm, cough) – Spontaneous breathing – Not requiring HFNC or tracheostomy – COVID-19 diagnosis ≥72 h	70/240 (29%)	Open-Label	140–180 ppm for 20–30 min, 2 times daily for 14 d	Reduction in incidence of requiring intubation and mechanical ventilation (within 28 d of treatment)
Beyond Air Inc. US Trial (NCT04397692)	Recruiting	Hospitalized COVID-19 patients: – Admitted within previous 24 h – SpO2 ≤ 93% – Shortness of breath (onset ≤8 d ago) – Not requiring HFNC, CPAP, intubation, mechanical ventilation, or tracheostomy – Not diagnosed with ARDS	20/20 (100%)	Open-Label	80 ppm for 40 min, 4 times daily	Time to deterioration measured by need for NIV, HFNC, or intubation (within 14 d of intervention)

ARDS = acute respiratory distress syndrome; BMI = body mass index; COVID-19 = coronavirus 2019 pandemic; ED = emergency department; HFNC = high flow nasal cannula; IBW = ideal body weight; ICU = intensive care unit; NIV = non-invasive ventilation; P_aO_2 = partial pressure of oxygen in arterial blood; RR = respiratory rate; S_pO_2 = peripheral oxyhemoglobin saturation; T2DM = type II Diabetes Mellitus. Note: Only trials within primary locations in the United States have been included. Details of 1 withdrawn study are excluded (NCT04398290).

6. Summary and conclusion

It should be clear that although there is reasonable physiological and biological rationale for iNO use in COVID-19, numerous important factors remain unanswered. Ongoing and future RCTs should aim to identify 1) predictive clinical indicators and biomarkers to best identify which patients are most likely to benefit from iNO treatment; 2) optimal timing of iNO initiation and duration of therapy; 3) best practices for dose selection and adjustment; and 4) which delivery methods may be best suited for iNO delivery in the context of COVID-19 patients. In light of the complex and heterogeneous nature of COVID-19, it is reasonable to surmise that a precision medicine or tailored approach taking into account a holistic view of each individual patient may be best in the application of iNO. In the absence of high-quality RCT data, current knowledge should be interpreted with caution. Individual patients should be considered on a case-by-case basis with sound clinical judgement from the attending physician.

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Declaration of competing interest

R-J.S. is an employee of Coherus BioSciences. The work described herein is solely reflective of the author's (R-JS) personal views and is unrelated to his job duties with Coherus BioSciences. These views do not constitute an endorsement by Coherus BioSciences, do not represent the views of Coherus BioSciences, and Coherus BioSciences had no role in the conception, writing, revision, or final approval of the manuscript. M. N.B. declares no conflicts of interest.

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