Short Communication

The effect of L-arginine supplementation on amelioration of oxygen support in severe COVID-19 pneumonia

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SUMMARY

Background & aims: L-Arginine (L-Arg) has been shown to help reduce respiratory support requirements in coronavirus disease 2019 (COVID-19), in an Italian study. We investigated the effect of L-Arg supplementation on the reduction in respiratory support for patients with severe COVID-19 pneumonia in an Indian population.

Methods: A parallel-group, triple-blinded, randomized controlled trial (RCT) was conducted on patients with severe COVID-19 pneumonia on oxygen (O_2) support. Patients received either 3 g of oral L-Arg or placebo, daily under supervision, until they were off O_2 support, or for a maximum of 10 days, whichever was earlier. The primary outcome was cessation in O_2 support. Other outcomes were time to cessation of O_2 support, duration of hospitalization, and incidence of adverse thrombotic events.

Results: We did an intention-to-treat analysis on 74 patients who were randomized into L-Arg (n = 38) or placebo group (n = 36). There were no significant differences between the two groups in the outcomes. At end of the study, 28 patients (73.6%) in L-Arg and 26 patients (72.2%) in the placebo group were weaned off oxygen support. The median number of days to the cessation of O_2 support estimated using Kaplan Meir survival analysis, was 3 days in the L-Arg group (95% confidence interval [CI]: 1.2, 4.7) and 5 days in the placebo group (95% CI, 4.1,5.8); P = 0.27.

Conclusion: In this group of patients with severe COVID-19 pneumonia, L-Arg supplementation did not show any significant difference in outcomes when compared to placebo supplementation.

1. Introduction

There has been a concerted effort globally to find treatment strategies to mitigate the severity of Coronavirus disease 2019 (COVID-19) infection. The effectiveness of anti-viral, immunomodulatory, and anti-inflammatory drugs remains uncertain due to large variations in the host response; therefore, the treatment strategy in COVID-19 remains a challenge [1]. Previous studies examining the host response in COVID-19 patients have reported altered amino acid (AA) metabolism and plasma AA profiles with significantly lower plasma arginine (Arg) concentrations in COVID-19 patients compared to healthy controls [2]. Lower Arg concentrations could contribute to endothelial dysfunction, hypercoagulability, and T-cell dysregulation, which are important features of COVID-19 disease progression [2,3]. In addition, Arg metabolism supplies nitric oxide (NO), which is one of the earliest antiviral responses to COVID-19 infection. NO also has a vasodilatory and antithrombotic effect, postulated to be protective in the hypercoagulable state in COVID-19 [4,5]. An interim analysis from an RCT on the effect of L-Arg in COVID-

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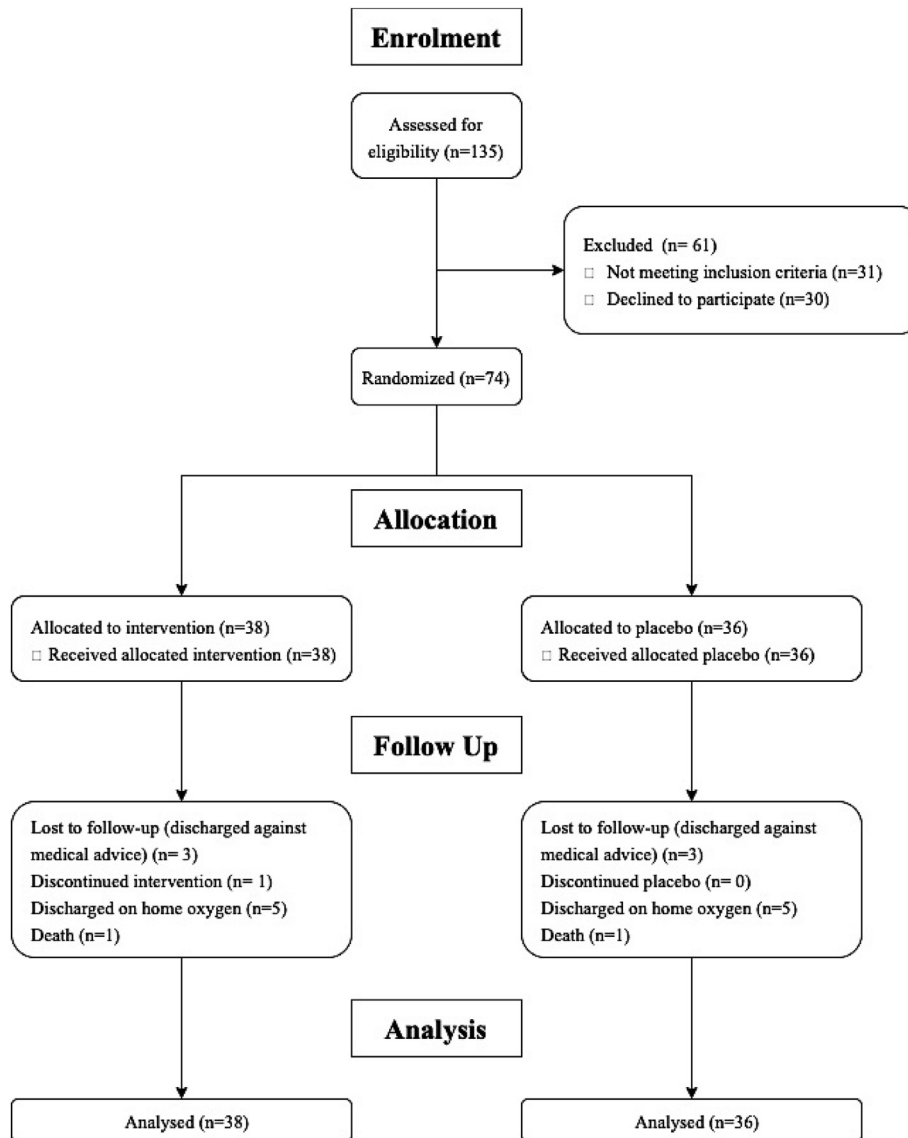*E-mail address:* a.kurpad@sjri.res.in (A.V. Kurpad).

Fig. 1. Screening, enrolment and allocation of participants.

19 showed that patients who received 1.66 g L-Arg twice daily, had a 6.6-fold reduction (P = 0.01) in their need for respiratory support compared to a placebo group [5]. This effect could be greater in populations with low Arg status, such as in India. We have earlier shown that South Indian women have lower Arg bioavailability associated with slower NO synthesis when compared to American and Jamaican women [6]. Therefore, this study tested the effect of LL-Arg supplementation on the need for respiratory support in patients with severe COVID-19 infection in India.

2. Materials and methods

An investigator-initiated, placebo-controlled, triple-blind, randomized controlled trial was conducted to assess the effect of L-Arg supplementation in patients admitted with severe COVID-19 pneumonia to a tertiary care center in South India. The trial was approved by Institutional Ethical Review Board (IERB) (CT 03/22) and registered prospectively in Clinical Trials Registry India (CTRI/ 2022/01/039549).

Hospitalized patients >18 y old were screened for SARS-CoV-2 with the Rapid antigen test (RAT) or reverse transcription polymerase chain reaction (RT-PCR). Those positive, with symptom onset <15 days prior, with severe COVID-19 pneumonia as defined by the National Institute of Health [7] with oxygen (O_2) saturation <93% when on ambient air (measured by pulse oximetry) or on supplemental O_2, and willing to consume L-Arg/placebo were included in the study. Patients with chronic lung disease who were on home O_2 therapy or required noninvasive ventilation (NIV) for an indication other than COVID-19 pneumonia, or on inotropic support at admission, or were unwilling to consume L-Arg/placebo were excluded. Written informed consent was obtained from the patient, or if they were unable to consent, from their legal representative.

Patients were randomized into the intervention or placebo arm in a 1:1 ratio based on a computer-generated randomization sequence. The randomization was maintained by a team that was uninvolved in the study and sequentially numbered sealed unlabeled color-coded opaque sachets of placebo or L-Arg were prepared. The enrolled patients, the treating clinicians, nursing staff, and outcome assessors were all blinded to the intervention. A Data and Safety Monitoring Board (DSMB) was constituted at the start and was regularly kept updated on all aspects of the study. The

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Table 1
Baseline characteristics.

Characteristic	ʟ-Arginine (n = 38)	Placebo (n = 36)
Age (years)[a]	61.7 ± 15.4	66.9 ± 9.1
Gender M/F (%)	63.1/36.8	55.6/44.4
Time to enrolment from symptom onset (days)[a]	7.0 ± 5.3	6.4 ± 4.7
Vaccinated with 2 doses (%)	84.6	90.5
DM (%)	42.1	72.2
IHD (%)	15.8	16.7
HTN (%)	44.7	66.7
Chronic Lung disease (%)	21.0	36.1
CKD (%)	10.5	16.6
Chronic Liver disease (%)	10.5	0
Neurological disease (%)	21.1	13.9
Connective tissue disorder (%)	7.9	5.6
Smoker (%)	24.2	24.1
WBC count $\times 10^9$ (n/L)[b]	4.39 (8.4,20.2)	3.07 (1.9, 12.5)
CRP (mg/L)[b]	9.03 (2.8,17.6)	6.22 (3.1, 14.7)
D-dimer (ng/mL)[b]	872.5 (531.5,2465.5)	572 (349.5,1063.5)
Ferritin (mcg/L)[b]	468 (124.6909.7)	267.8 (94.9779.2)
Steroids (%)	81.6	86.1
Remdesivir (%)	18.4	16.7
Oxygen support at baseline (L/min)[a]	4.0 ± 2.07	4.9 ± 2.35

No significant differences observed.
DM: Diabetes Mellitus; IHD: Ischemic heart disease; HTN: Hypertension; CKD: Chronic kidney disease; WBC: White blood cell; CRP: C Reactive Protein.
[a] Mean ± SD.
[b] Median (IQR: 25th percentile, 75th percentile).

patients in the intervention arm received 3 g of ʟ-Arg in a 5 g powdered formulation (Argin, Nutrigrow, India). Patients in the placebo arm received 5 g of flavored glucose (Glucon-D Orange, Zydus Wellness Product Ltd, India), with a similar appearance (color, smell, and taste) to the intervention. This was administered orally as a solution in 100 mL of water, made up by study staff, followed by a 100 mL water rinse. The patients were administered the intervention daily for 10 days or until they were off O_2 support, whichever was earlier. The compliance to the intervention, daily O_2 support (L/min), O_2 delivery device used, adverse events during the intervention, and length of hospital stay was recorded. The primary outcome was complete cessation of O_2 support. Secondary outcomes were the number of days to the cessation of O_2 support, length of hospital stay post-enrollment, reduction in the frequency of thrombotic events, and in-hospital mortality. The required sample size, based on a 20% (absolute) greater number with cessation of O_2 support in the ʟ-Arg group compared to the placebo group [5], with 80% power and 5% level of significance, was 202 patients.

Continuous observations were reported as mean ± standard deviation (SD) or Median with interquartile range based on their distribution, and categorical variables were reported as percentages. Grouped data were compared using two-sample t-test or Mann-Whitney U test for continuous variables, based on their distributions and Chi-Square or Fischer Exact test for categorical variables. Intention to treat analysis was performed and differences in the group-wise median time for cessation of O_2 support, and days to discharge, were estimated by Kaplan Meir analysis, and compared using Log rank test. A P-value < 0.05 was considered to be statistically significant for all comparisons. All analyses were performed using IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

3. Results

The study was conducted between January 2022 and February 2022, during the third wave of COVID-19 infection in South India [8]. Patient screening and enrollment are described in Fig. 1.
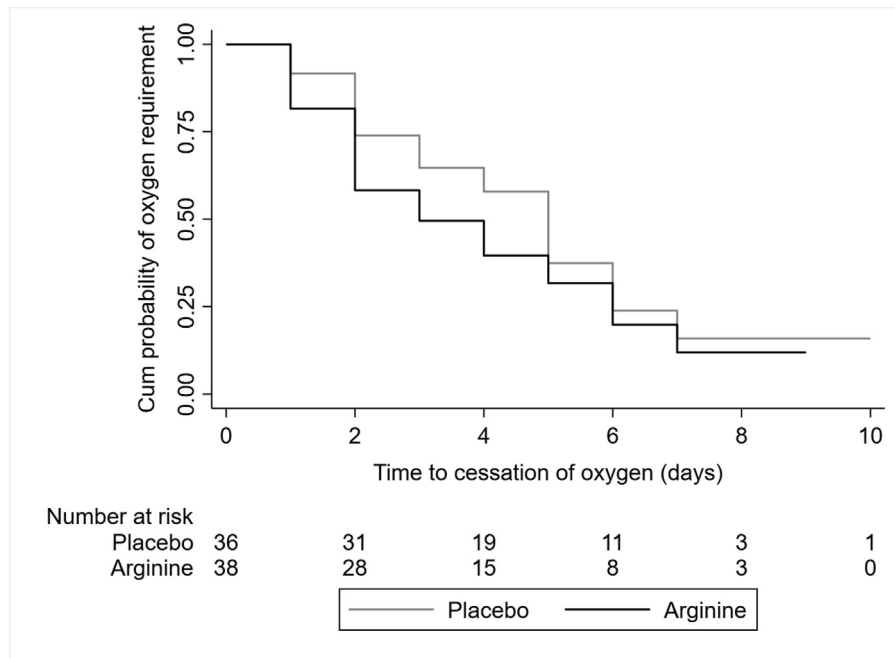
A total of 135 patients were screened by the time this wave had receded, of which 74 patients fulfilled the inclusion criteria and had consented to participate. After randomization, 38 were allotted to the ʟ-Arg and 36 to the placebo group. The baseline characteristics of the enrolled patients are presented in Table 1.

An intention-to-treat analysis was performed. All patients at baseline were on oxygen support by facemask or nasal prongs. At the end of the study, oxygen support ceased in 28 patients (73.6%) in ʟ-Arg and 26 patients (72.2%) in the placebo group. On the other hand, 3 patients in the ʟ-Arg group (NRBM-2, Mechanical Ventilation-1), and 8 patients in the placebo arm (NRBM-3, NIV-4, Mechanical ventilation-1) required higher O_2 support during hospitalization. The median number of days to O_2 cessation in the first 10 days of follow-up was 3 in the ʟ-Arg group (95% confidence interval [CI]: 1.2, 4.7) and 5 in the placebo group (95% CI: 4.1, 5.8); P = 0.27 (Fig. 2A). The median time to discharge from enrollment to the study was 6 days in the ʟ-Arg group (95% CI: 4.4, 7.6) and 7 days in the placebo group (95% CI: 4.4, 9.6); P = 0.42 (Fig. 2B).

Eight patients, aged between 44 y to 78 y, 4 women and 4 men had non-ST elevation myocardial infarction (NSTEMI) during the study period (3 (8%) in ʟ-Arg and 5 (14%) in placebo). In addition, one patient a 38 y old man who received ʟ-Arg, had a pulmonary thromboembolism (PTE) during his hospitalization (3% of ʟ-Arg arm). Five patients had worsening in their clinical status and were transferred to the intensive care unit (2 (5%) in ʟ-Arg, and 3 (8%) in placebo). Of these 5 patients, two (55 y old woman in placebo arm and 73 y old man in ʟ-Arg arm) died during the study period due to worsening COVID-19 and sepsis respectively (1 (3%) in each arm). These serious adverse events (SAE) were deemed to be unrelated to the study by the DSMB and the IERB and no significant difference was observed between the placebo and ʟ-Arg arms (P > 0.05).

4. Discussion

The present study was conducted to evaluate the effect of ʟ-Arg supplementation on the reduction in respiratory support for patients with severe COVID-19 pneumonia in an Indian population. Previous studies have demonstrated a state of acute Arg depletion

A. Kaplan Meir curves assessing the time to cessation of O$_2$

Number at risk

Placebo	36	31	19	11	3	1
Arginine	38	28	15	8	3	0

B. Kaplan Meir curves assessing the length of in-hospital stay from enrolment

Number at risk

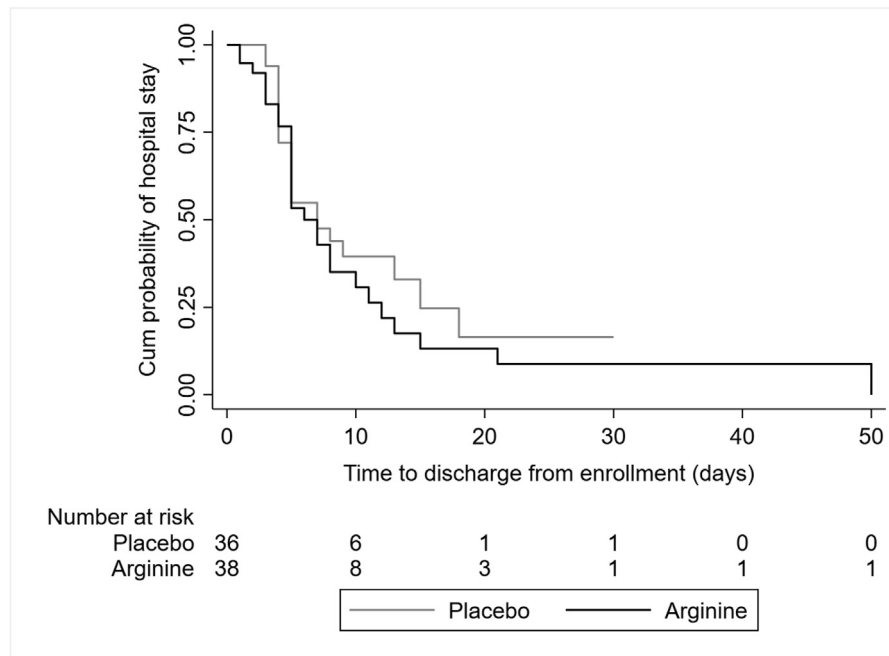|						
Placebo	36	6	1	1	0	0
Arginine	38	8	3	1	1	1

Fig. 2. A. Kaplan Meir curves assessing the time to cessation of O$_2$. B. Kaplan Meir curves assessing the length of in-hospital stay from enrolment.

with low plasma Arg concentration in COVID 19 patients compared to healthy controls [2]. This depletion is hypothesized to contribute to T cell dysregulation, endothelial dysfunction and coagulopathy, through arginase upregulation [2]. Higher arginase expression could suppress T cells and promote viral replication in COVID-19 infection [9]. Arg deficiency also leads to increased platelet

adherence and decreased NO production, leading to vasoconstriction and hypercoagulability [10].

The provision of L-Arg resulted in no statistically significant difference for any respiratory outcome in patients with severe COVID-19 infection. These findings contrast with an earlier Italian study, in which L-Arg supplementation significantly reduced

respiratory support by 6.6-fold in patients with severe COVID-19 at 10 days [5]. Despite the sample size in the Italian study being comparable to the present study (74 patients in our study versus 90 patients in the Italian study), the effect of L-Arg was not replicated in our study. This could be because of several reasons. Compared to the present study, patients in the Italian study were sicker, required higher oxygen support at baseline, and were hospitalized for longer, which could be attributed to the different SARS-COV-2 variants in the two studies, with the earlier δ (delta) variant in the Italian study being more severe compared to the later ο (omicron) variant in the present study [8]. Additionally in that study, the patients in the L-Arg group were sicker compared to the placebo group, which might have resulted in a quicker transition to lower O_2 support in the L-Arg group [5]. It is also possible that the dose of L-Arg administered in our study could have been subtherapeutic due to the lower L-Arg bioavailability in the Indian population as has been shown in previous literature [6]. Another multicentre study from Italy, on Long COVID-19, showed that patients who received L-arginine supplementation with Vitamin C, improved symptomatically with better effort perception, compared to those on multivitamins [11]. This should be replicated in different populations before definitive conclusions can be drawn. In addition, L-Arg in higher doses should be tested in the Indian population for COVID-19 infection.

A limitation was the inability to achieve the intended sample size, due to the rapid tailing off, of this COVID-19 wave [8]. A statistical power recalculation showed that the recruited number of patients could only have observed a difference of 32% (as opposed to the 20% difference used in the original sample size calculation) in the number of patients with cessation of O_2 support in the intervention group compared to placebo, and thus a type II error may have been present. In conclusion, there was no significant effect of L-Arg supplementation on the oxygen requirement of patients with severe COVID-19 infection.

Funding

Author contribution

All authors took part in conceptualization, clinical conduct of the study, data curation, analyses and drafting of the paper. JMR, TT were responsible for statistical analyses.

Declaration of competing interest

No conflict of interest to declare.

Acknowledgments

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