Double-blind, randomized, placebo-controlled trial with N-acetylcysteine for treatment of severe acute respiratory syndrome caused by COVID-19

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Summary

We designed a double-blind, randomized, placebo-controlled trial to determine whether high-dose N-acetylcysteine could prevent respiratory failure in patients with severe acute respiratory syndrome caused by Covid-19; however, no difference was observed between the placebo and experimental groups.

ABSTRACT

Background: A local increase in angiotensin 2 after inactivation of angiotensin-converting enzyme 2 by SARS-CoV-2 may induce a redox imbalance in alveolar epithelium cells, causing apoptosis, increased inflammation and, consequently, impaired gas exchange. We hypothesized that N-acetylcysteine (NAC) administration could restore this redox homeostasis and suppress unfavorable evolution in Covid-19 patients.

Objective: To determine whether NAC in high doses can avoid respiratory failure in patients with Covid-19.

Methods: It was a double-blind, randomized, placebo-controlled, unicentric trial, conducted at the Emergency Department of Hospital das Clínicas, São Paulo, Brazil. We enrolled 135 patients with severe Covid-19 (confirmed or suspected), with an oxyhemoglobin saturation of less than 94% or respiratory rate higher than 24 breaths/min. Patients were randomized to receive NAC 21 g (approximately 300 mg/kg) for 20 hours, or dextrose 5%. Primary endpoint was the need for mechanical ventilation. Secondary endpoints were time of mechanical ventilation, admission to ICU, time in ICU, and mortality.

Results: Baseline characteristics were very similar in the two groups, with no significant difference in age, sex, comorbidities, medicines taken, and disease severity. Also, groups were similar in laboratory tests and chest CT scan findings. Sixteen patients (23.9%) in the Placebo group were submitted to endotracheal intubation and mechanical ventilation, compared to 14 patients (20.6%) in the NAC group (p=0.675). No difference was observed in secondary endpoints.

Conclusion: Administration of NAC in high doses did not affect the evolution of severe Covid-19.

Key Words: Pneumonia; Mechanical ventilation; Angiotensin

INTRODUCTION

Severe acute respiratory infection (SARI) caused by the SARS-CoV-2 virus may lead to lung failure and the necessity of mechanical ventilation [1–3].

The mechanisms by which the virus affect the alveolar epithelia are not entirely understood [4]; however, it seems that, upon reaching the lower airways, the virus spike protein binds to the angiotensin-converting enzyme 2 (ACE2) and utilizes it as a means to enter the alveolar cells [5].

ACE2 is an enzyme that catalyzes the conversion of angiotensin II (AngII) in angiotensin 1-7 [6], and it appears to be inactivated by the action of the virus. There is still no robust experimental confirmation for this fact; however, increased serum AngII levels have been reported in patients with severe Covid-19 cases [7] and enhanced expression of ACE2 in adults, compared to children, has been advocated to cause the difference of disease prevalence in these age ranges [8].

Physiological intracellular signaling of AngII involves increased production of reactive oxygen species (ROS), either through the activity of the Nox enzyme [9] or mitochondria [10]. At first, these ROS are used in signaling mechanisms; however, in excess, they can lead to cell apoptosis or necrosis [11].

Moreover, vascular smooth muscle cells in culture exposed to AngII enhance the expression of CD40, a crucial mediator of the acquired immune response [12]. This phenomenon occurs through a redox-signaling pathway, involving enhanced Nox expression and hydrogen peroxide generation. In these experiments, AngII-induced CD40 expression was blocked by treatment with N-acetylcysteine (NAC) [12].

NAC has been in clinical use as a mucolytic since the 1960s [13]. It is also currently used in acute liver failure [14] or acetaminophen poisoning [15]. Its safety is well documented, and its effectiveness in lung diseases may be beyond its mucolytic action, since it may also interfere with the inflammatory response and bronchial tone [16].

NAC may also replenish intracellular reduced glutathione (GSH) pools by providing cysteine, an essential precursor in GSH synthesis [17]. Therefore, NAC administration could restore intracellular redox signaling through enhanced activity of the reduced-oxidized glutathione pair (GSH-GSSG), the primary intracellular antioxidant system [18].

We hypothesize that the local increase in AngII after inactivation of ACE2 by SARS-CoV-2 leads to a redox imbalance in the alveolar epithelium cells, causing apoptosis, breaking of the alveolar-capillary barrier and, consequently, impaired gas exchange and respiratory failure. Other authors also raised this hypothesis [19].

Therefore, we designed a double-blind, placebo-controlled, randomized clinical trial to determine whether NAC (in doses used to treat acute liver failure), is able to protect alveolar cells and avoid respiratory failure in patients with severe acute respiratory syndrome caused by (confirmed or suspect) Covid-19.

METHODS

This is a double-blind, randomized, placebo-controlled trial to assess the effectiveness and safety of intravenous NAC to prevent respiratory failure in patients with confirmed or suspect severe Covid-19.

It was a unicentric trial, conducted at the Emergency Department of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo in São Paulo, Brazil. The Institutional Ethics Committee approved this trial under #30420720.4.0000.0068, and it was registered at the Brazilian Registry of Clinical Trials (REBEC) under # UTN: U1111-1250-356. The approved protocol can be assessed at the REBEC site (http://www.ensaiosclinicos.gov.br/rg/RBR-8969zg/). Written informed consent was obtained from all patients or their legal representatives if they were unable to provide consent.

Patients

Patients 18 years of age or older diagnosed with severe Covid-19 (suspected or confirmed) were eligible for the study. Inclusion criteria were oxyhemoglobin saturation (SaO2) of less than 94% while breathing ambient air and respiratory rate higher than 24 breaths/min. We chose 24irpm because that is the criteria for admission to our hospital

Exclusion criteria included known allergy or hypersensitivity to NAC, signs of the imminent need for orotracheal intubation (increased respiratory effort, decreased level of consciousness, SaO2 < 90% with supplemental oxygen), pregnancy or refusal to sign the written consent.

Trial Design

The trial was initiated in rapid response to the Covid-19 public health emergency, at which time there was minimal information about clinical outcomes in hospitalized patients with Covid-19.

The trial was conducted from April 10, 2020, to May 25, 2020 (date of the last patient enrollment), at the Emergency Department of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Eligible patients were randomly assigned (1:1) to either the NAC or the Placebo groups. Randomization was performed by the Chief Pharmacist, using QuickCalcs - Random number calculators (© 2018 GraphPad Software, LLC; San Diego, CA), a free website tool. According to the sequential order of the randomization center, eligible patients were allocated to receive medication in individually numbered packs. The Chief Pharmacist kept the code for the records until the end of the study.

Study Protocol

NAC was prescribed according to a "two-bag" protocol, as proposed by Wong et al. for paracetamol poisoning [20].

Twenty-one grams of NAC were administered intravenously for each patient in the NAC group, divided into two doses: 14 g in the first 4 hours and 7 g in the next 16 hours. NAC was diluted in dextrose 5%, 500ml. Therefore, the concentration for each dose was 28 mg/ml, and 14 mg/ml, respectively, and the amount of volume received for each patient was 1000 ml in 20 hours. Patients in the placebo group received the same amounts of dextrose 5% in water (1.000 ml in total) intravenously.

All patients received standard care, according to the institutional protocol. At the Emergency Department, it included oxygen supplementation, invasive ventilation, and antibiotics.

All patients received the drugs at the Emergency Department and were later forwarded to hospital wards, ICUs, or discharged, according to the in-charge Emergency Physician discretion.

All patients in this study received empirically Ceftriaxone 2g / day and Azithromycin 500mg / day, which was the protocol in our Emergency Department for severe Covid-19.

Laboratory and Radiological tests

Routine laboratory tests were collected from all patients included in the study, including white blood cell counts, C-reactive protein, renal function, lactate dehydrogenase, electrolytes and D-dimer.

We confirmed Covid-19 by reverse-transcriptase polymerase chain reaction (RT-PCR) assay of nasopharyngeal swab or tracheal aspiration specimens.

Chest computed tomography was also obtained for all patients. Lung involvement extension was classified as higher or lower than 50% of lung volume.

Outcomes

The primary clinical endpoint was the necessity for intubation and invasive mechanical ventilation.

Secondary outcomes were time of invasive mechanical ventilation, admission to ICU, time of ICU internment, and mortality.

Statistical analysis

The original total sample size was set at 140, since it would provide the trial with 80% power to detect a reduction in the need for invasive mechanical ventilation of 50%, at a two-sided significance level of α=0.05.

On April 30, 2020, the data and safety monitoring board reviewed results. This review was initially planned as an interim analysis. The planned enrollment of 100 patients in the trial occurred quickly, and the assessment at that point was that the trial was underpowered; thus, a decision was made to continue enrollment by investigators.

Primary efficacy analysis was on an intention-to-treat basis and included all the patients who had undergone randomization.

Because the statistical analysis plan did not include a provision for correcting for multiplicity in tests for secondary or other outcomes, results are reported as point estimates and 95% confidence intervals. The widths of the confidence intervals have not been adjusted for multiplicity, so the intervals should not be used to infer definitive treatment effects for secondary outcomes. Safety analyses were based on the patients' actual treatment exposure.

Age and other continuous variables are presented as median and interquartile range (IQR). Comparison between groups was evaluated by the chi-square test for categorical variables, Wilcoxon test for non-parametric variables and Students t-test for the others.

RESULTS

During the study period, 612 individuals were assessed for eligibility, and 140 were chosen and randomized (Figure 1).

From the 140 sets prepared by the pharmacy, two bags were mishandled during administration, and three patients lost their drug packs while been transferred inside the hospital. Therefore, at the end of the study, we had 67 patients in the placebo group and 68 patients in the NAC group. These 135 patients were included in the final analysis (Table 1)

Baseline characteristics were very similar between the two groups. The median age was almost identical, as well as the incidence of comorbidities. We present the two most prevalent (hypertension and diabetes), but other diseases, like cancer and autoimmune diseases, were also identical in both groups.

Of the 63 hypertensive patients, 30 (47.6%) used angiotensin-receptor blockers. These patients were divided equally into both groups (14 of 32 in the Placebo group and 16 of 31 in the NAC group).

The time between the first symptoms of the disease and the protocol inclusion for patients in the NAC group was seven days (IQR: 5-10), while for the Placebo group was also seven days (IQR: 6-11.5).

At the time of hospital admission, oxyhemoglobin saturation was equal in both groups, and all patients needed oxygen supplementation upon arrival at the Emergency Department. We assessed disease severity utilizing the seven-category ordinal scale, proposed by the World Health Organization [21]. The vast majority of patients (99.3%) were classified in categories 4 and 5, hospitalized, and about 68% requiring oxygen supplementation or non-invasive ventilation and ongoing care. The severity categories in both groups were, again, very similar.

Laboratory findings were also similar in both groups. We show in Table 1 the values of circulating leukocytes and C-reactive protein. Other exams reportedly increased in Covid-19 (LDH, D-dimer) were also equal in both groups.

Finally, SARS-CoV-2 detection has been observed in 63 (94.0%) in the Placebo group and 65 (95.6%) in the NAC group.

In summary, the placebo and the experimental groups were equal, suggesting that our randomization process was very effective.

Outcomes

Patients assigned to the NAC group did not differ in the necessity of invasive mechanical ventilation compared to patients assigned to the Placebo group in the intention-to-treat population. From the Placebo group, 16 patients (23.9%) were submitted to endotracheal intubation and invasive mechanical ventilation. The number of patients in the NAC group was 14 (20.6%) (Table 2).

Mortality in both groups was almost equal: 10 patients in Placebo (14.7%) and 9 in NAC (13.4%). At the end of the study, six patients were still in ICU, three in each group. These patients were not included in mortality analysis.

The need for ICU admission was 42.3% in Placebo, and 47.1% in NAC, values that were not statistically significant. Placebo patients remained in ICU for 8 days, while patients who received NAC remained for 9 days.

No difference was observed time of hospitalization between the two groups.

When only the 128 patients with positive RT-PCR for SAR-CoV-2 were included, the results were the same.

No adverse effect was observed in patients who received NAC. All patients tolerated the drug and the volume well.

In summary, NAC administration did not affect the evolution of SARS caused by Covid-19.

DISCUSSION

The evolution of Covid-19 to respiratory failure involves a complex net of inflammatory and vascular events [4].

We hypothesized that the increased availability of AngII caused by the SARS-CoV-2 virus-induced ACE2 blockade could be a crucial component in the disease pathogenesis. Considering that AngII signals inside the cell through a redox mechanism, we designed this clinical trial, aiming to block the intracellular action of AngII and prevent alveolar cells inflammation and apoptosis.

This was a double-blind, placebo-controlled, randomized trial, where 135 patients diagnosed with severe acute respiratory infection were enrolled. The baseline characteristics of the two groups were very similar in all aspects.

Regarding the outcome, there was no difference between the Placebo and NAC groups neither in the primary outcome (need for invasive mechanical ventilation) nor the secondary ones (mortality, ICU admission, time of invasive mechanical ventilation).

Several papers have been published advocating that redox imbalance caused by AngII could be a crucial point in SARS-CoV-2 pathogenesis [20,22,23]. Our data do not support these hypotheses.

It is necessary, therefore, to discuss the causes of this treatment failure in improving respiratory function in Covid-19 patients.

First, we have to speculate whether the drug and dosage proposed to restore redox balance in these patients were the right ones. Since our objective was to correct intracellular redox imbalance, NAC was a natural choice, for acting on the reduced-oxidized glutathione pair, the central intracellular antioxidant system [12,18,24]. Moreover, NAC has shown the ability to restore the intracellular redox imbalance in vitro experiments [12,25,26] and has been safely prescribed in patients with acute liver failure [14] and acetaminophen poisoning [15].

Regarding the posology, preparing individual doses (300 mg/kg, as recommended in these studies) would be time-consuming, and we had a small window for drug administration. Hence, we chose a fixed dose (21 g), an average for the recommended dose [20]. Although we did not measure patients' weight, we did not include very obese patients. Therefore, we believe that all patients received approximately 300 mg/kg, a dose even higher than the ones that have been used successfully in other clinical conditions [27,28].

The second hypothesis for the failure is related to the time of disease when NAC was administered. We chose patients with severe Covid-19 since we believed that in patients already in respiratory failure, the inflammation could not be controlled acting on redox signaling [29]. All included patients had pulmonary infiltrates in chest CT scan and needed oxygen supplementation, which suggests they already had substantial lung involvement. We can speculate that earlier NAC prescription could be more effective at the start of the symptoms; however, we know that most of these patients would recover spontaneously [30]. It would not be feasible or ethical to treat these patients for 20 hours in a hospital, knowing that most of them would not need it.

Finally, we cannot exclude the fact that our initial hypothesis was wrong; that is, excess AngII neither leads to intracellular redox imbalance nor influences the disease pathogenesis. It is documented that there is an increase in AngII in severe Covid-19 patients

[7]; however, it can be just a collateral phenomenon, and it has nothing to do with the disease pathophysiology. Against this hypothesis is the fact that published data [31,32], and our own series of cases (submitted to publication), show a better prognosis for patients in the use of angiotensin receptor blockers. These data suggest that AngII may be involved, someway, in SARS-CoV-2 pathogenesis.

The main limitation of our study is the small number of patients enrolled. This protocol has been initially proposed as a preparatory stage before a larger multicentric trial; however, given the unambiguous negative results, the larger study was aborted.

Nevertheless, several opinion articles continue to propose acting on AngII [33] or redox signaling [34,35] as a viable therapeutics for Covid-19. That is the importance of our work. We have shown here that the administration of NAC in high doses did not affect the evolution of severe Covid-19.

The question remains on how to act on these signaling pathways activated by AngII without impairing patients' hemodynamics and renal function. We believe that interactions between AngII and inflammation should be better studied in the near future and can probably furnish new ideas for treating Covid-19.

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Authors´ contributions:

JCGA, JFM, LOM, RABN: inclusion of patients, data collection and analysis, writing the paper

CLM: research nurse responsible for administering the drugs and monitoring patients

ADM, LMGG, FL, CGB, MCSM: statistical analysis, filling the data spreadsheets

MAF, EAS, MFSM, VBP, CEC: pharmacists responsible for preparing the medication, blinding and randomization

HPS: proposed the study, final data analysis, final writing

Covid Register Group: collecting the data

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Figure 1. Randomization and Treatment Assignment

(Insert Figure 1)

Table 1. Demographic, Clinical, Laboratory, and Radiological Findings at Baseline

Characteristics	**N-acetylcysteine (N = 67)**	**Placebo (N = 68)**	**p**
Age, median (IQR), y	59 (47-70)	58 (48-70)	0.996
Male sex - no. (%)	43 (67)	37 (54)	0.248
COVID confirmed - no. (%)	65 (97)	63 (93)	0.737
Days from symptoms onset to randomization – median (IQR),	7 (5-10)	7 (6-11.5)	0.435
Coexisting conditions - no. (%)			
Hypertension	32 (47)	31 (45)	0.800
Diabetes	29 (43)	22 (32)	0.190
Cancer	8 (12)	9 (13)	0.791
Autoimmune disease	3 (4)	2 (3)	0.604
Previously use of ACE inhibitor	5 (7)	7 (10)	0.563
Previously use of angiotensin II receptor blocker	14 (21)	16 (23)	0.713
Fever - no. (%)	37 (55)	33 (48)	0.436
Respiratory rate, median (IQR), breaths/min	26 (22-32)	25.5 (21.5-30)	0.647
Oxyhemoglobin Saturation, median (IQR), %	91 (89-93)	91 (89-92.5)	0.771
White-cell count ($x10^{-9}$/liter) - median (IQR)	8385 (5365-10262)	7345 (6265-9017)	0.452
C-reactive protein, median (IQR), mg/dl	110 (77.5-156.5)	83 (45-179.5)	0.130

Lactate dehydrogenase, median (IQR), mg/dl	354.0(268.0–472.2)	397.0 (319.0-478.1)	0.0578
D-dimer, median (IQR), mg/dl	1058.0(588.1-2195.2)	1070.3(643.0-2291.1)	0.959
sCD40L, median (IQR), ng/dl	4.16(3.06-6.67)	5.51(4.00-8.22)	0.406
Radiologic findings on chest CT scan* - no (%)			
< 50% of lung involvement	45 (67%)	44 (65%)	0.855
Score on ordinal scale**- no. (%)			
4. Hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19-related)	20 (30)	22 (32)	0.796
5. Hospitalized, requiring supplemental oxygen	46 (68)	46 (68)	0.850
6. Hospitalized, receiving noninvasive ventilation or high-flow oxygen devices	1 (1)	0 (0)	0.306

*Ground-glass opacity infiltration or consolidation

** According to WHO criteria

Percentages may not total 100 because of rounding. IQR denotes interquartile range.

Table 2. Outcomes in the intention-to-treat population

Characteristic	**N-acetylcysteine (N = 67)**	**Placebo (N = 68)**	**Difference***	**p****
Invasive mechanical ventilation – no (%)	16 (24)	14 (21)	1.21 (0.53-2.72)	0.641
Death - no (%)	9 (14)	9 (14)	1.03 (0.38-2.82)	0.940
ICU admission	29 (43)	32 (47)	0.85 (0.43-1.69)	0.652
ICU stay — median no. of days (IQR)	9 (5-14)	8 (4-15)		0.557
Hospital stay — median no. of days (IQR)	11 (5.5-19)	10 (7-16.5)		0.872

*Odds ratio (Confidence interval)

Figure 1