

Clinical Trial Assessment of Intermittent and Continuous Infusion Dose of N-Acetylcysteine on Redox Status of the Body in Patients with Sepsis Admitted to the ICU

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Abstract

Purpose: Conflicting results exist regarding the efficacy of N-acetyl cysteine (NAC) in sepsis treatment. A pivotal factor affecting the therapeutic potency of NAC in sepsis is timing and dosing of its infusion. We aimed to assess the effect of NAC on redox status of patients with sepsis and to compare its efficacy in intermittent and continuous infusion with the objective of developing the infusion regimen and optimizing the timing. **Materials and Methods:** A prospective, randomized clinical trial was designed to compare the antioxidative effect of NAC in intermittent infusion group (IV: 25 mg/kg bolus and then 25 mg/kg/8 hours 3 times) and continuous infusion group (IV: 25 mg/kg bolus and then 75 mg/kg over 24 hours) in 60 critically ill patients with sepsis (20 patients in each group). Blood samples were collected immediately before and after intervention for total antioxidant capacity (TAC) and malondialdehyde (MDA) assessment. **Results:** N-acetyl cysteine considerably increased TAC levels in both intermittent (0.68 ± 0.60 ; P value = .036) and continuous (0.69 ± 0.64 ; P value = .015) infusion groups when compared to placebo (0.61 ± 0.10); however, the difference in TAC levels between the intermittent and the continuous infusion did not reach statistical significance (P value = .942). Likewise, NAC treatment decreased MDA levels in both intermittent (19.45 ± 4.18 ; P value = 0.001) and continuous (22.47 ± 6.68 ; P value = .002) infusion groups when compared to placebo (31.76 ± 11.06), while the difference in MDA levels between the intermittent and the continuous infusion did not reach statistical significance (P value = .481). **Conclusion:** Our data confirmed the antioxidative effect of NAC treatment in patients with sepsis, with no significant difference in intermittent and continuous infusion.

Keywords

sepsis, N-acetyl cysteine, oxidative stress, antioxidant

Introduction

Sepsis is a medical condition caused by overwhelming body responses to severe infections.¹ It can lead to systemic inflammatory responses, tissue damage, organ failure, and eventually septic shock.² Despite numerous advances in medications and therapies, sepsis, with the mortality rate of 30% to 50%, continues to be one of the major reasons for death in intensive care units (ICUs).³ As the uncontrolled immune responses alongside altered redox status have been shown to be closely tied to the disease pathogenesis, medication approaches have mainly focused on the administration of anti-inflammatory and antioxidant agents as an adjunct to conventional therapies.^{4,5} It is believed that inhibition of excessive inflammatory mediators as well as reactive oxygen species could assist in providing an efficient, protective, and therapeutic treatment for the disease.⁶⁻⁹

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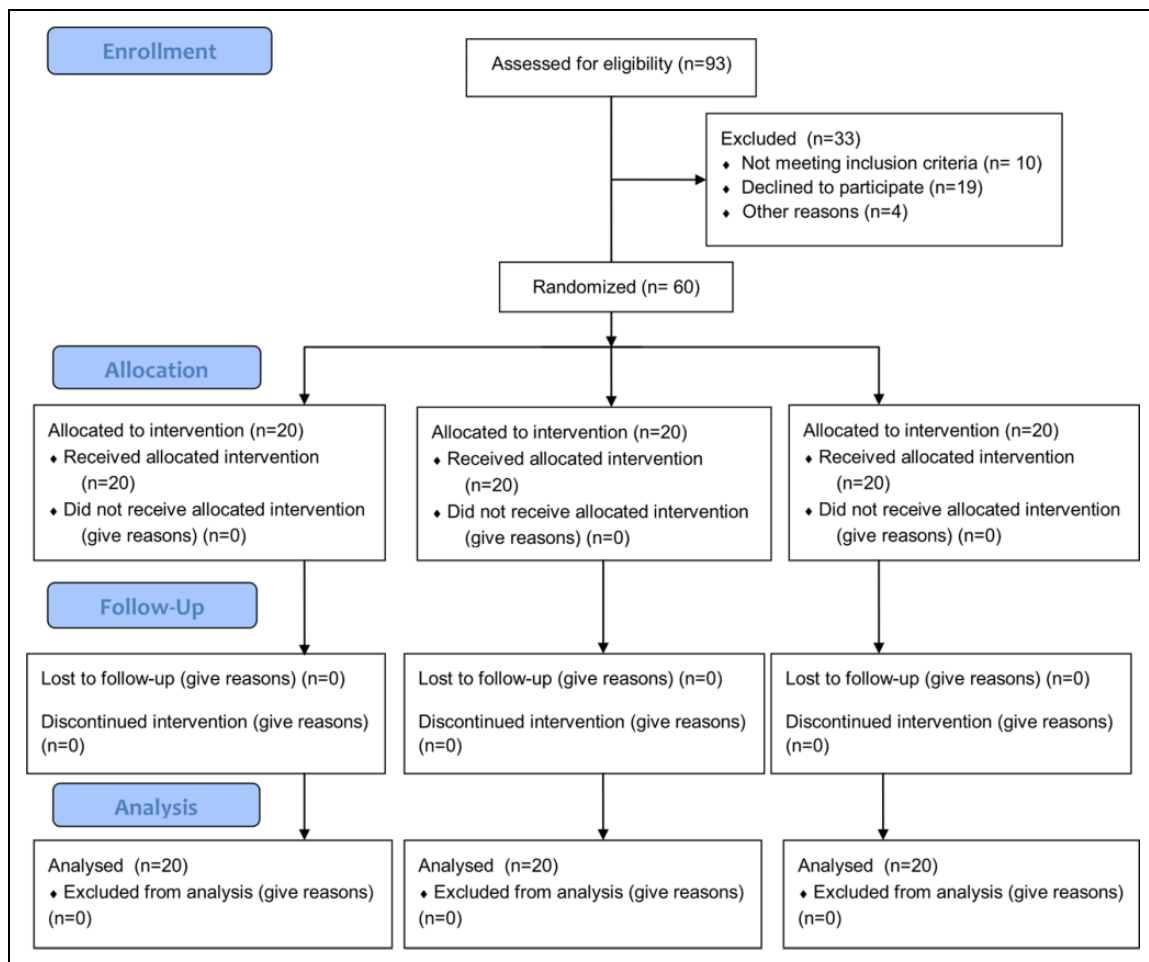


Figure 1. The flowchart of this clinical trial.

Among the wide range of drugs and chemicals routinely used for the treatment of the disease, N-acetylcysteine (NAC) has been referred as one of the most potential ones possessing both anti-inflammatory and antioxidative properties. It has been addressed as a safe drug with protective effects in septic condition.¹⁰⁻¹⁴ Moreover, it exerts vasodilatory functions that could show benefit in microcirculation in sepsis.⁷ Coherently, it was observed that NAC improves the oxygenation and lung compliance in patients with early diagnosed septic shock. Furthermore, the NAC-treated group had a shorter stay in the ICU.¹⁵ However, several other contradictory findings have been published regarding some drawbacks of its clinical utility in sepsis therapy. For instance, it was found that patients with severe sepsis did not show any statistical significance in outcomes following NAC treatment.¹⁶ According to these contrary data, it has been suggested that a pivotal factor affecting the efficacy of NAC is the timing and dosing of its infusion. Thus, utilizing an appropriate prescription of NAC administration appears to be of vital importance. Therefore, in the current study, we first aimed to assess the effect of NAC on the redox status of the patients with sepsis and then compare its efficacy in intermittent and continuous infusion with the objective of developing the infusion regimen and optimizing the timing.

Materials and Methods

Ethical Statement

This prospective, randomize clinical trial was approved by the Research and Ethics Committees of Mashhad University of Medical Sciences. The written informed consent was obtained from all participants or their relatives.

Study Population

Sixty critically ill patients with sepsis admitted to the ICU at Imam Reza Hospital were recruited in this pilot study (Figure 1). All the patients fulfilled 2 factors of systemic inflammatory response syndrome criteria as outlined earlier.³ Patients who were receiving supplements such as vitamins with antioxidant properties known as C, E, A, trace elements including zinc, manganese, selenium, and NAC before entering the study and those with NAC drug contraindications for any reason were excluded.

Intervention

Patients were randomly assigned to 3 groups with using a random number table as follows: (1) Intermittent infusion group

Table 1. Baseline Characteristics: Demographic Characteristics of the Participants.^a

Characteristics	Status	Placebo, n = 20	Intermittent, n = 20	Continuous, n = 20	P Value
Age, years		56.2 ± 16.9	53.9 ± 18.4	63.7 ± 14.2	.159
Sex, abundance (%)	Male	8 (40%)	8 (40%)	9 (45%)	.934
	Female	12 (60%)	12 (60%)	11 (55%)	
Ventilation, abundance (%)	+	13 (65%)	14 (70%)	15 (75%)	.89
	–	6 (30%)	5 (25%)	5 (25%)	
SAPS		40.9 ± 15.2	43.7 ± 17.1	47.0 ± 13.6	.656
Mortality risk prediction		32.6% ± 15.2%	37.7% ± 27.9%	41.0% ± 26.0%	.661

Abbreviations: SAPS, Simplified Acute Physiology Score; SEM, standard error of the mean.

^aData are expressed as mean ± SEM and number (%).

received 25 mg/kg intravenous bolus NAC at the beginning and then 25 mg/kg every 8 hours for 24 hours (total 100 mg/kg during the 24 hours), (2) continuous infusion group received 25 mg/kg intravenous bolus NAC at the beginning and then 75 mg/kg intravenous NAC as a continuous infusion over 24 hours (total 100 mg/kg during the 24 hours), and (3) placebo group received 100 mL/kg isotonic saline. The difference in age (P value = .159) and sex (P value = .934) in the 3 mentioned groups was not statistically significant.

Demographic information including age and sex and patients' respiratory state was collected on the arrival to the ICU. The prediction of mortality risk was calculated based on Simplified Acute Physiology Score (SAPS) score. Blood samples were collected immediately before and after the intervention and were centrifuged, the sera were extracted, and stored at -20°C for total antioxidant capacity (TAC) and malondialdehyde (MDA) assessment, 2 well-known markers for evaluation of oxidative status.

Thiobarbituric Acid Reactive Substances Assessment

The assay procedure was performed according to the manufacturer's protocol (Thiobarbituric Acid Reactive Substances assay kit-10009055; Cayman, Ann Arbor, Michigan). Briefly, this method is based on the reaction between MDA and thiobarbituric acid under high temperature and acidic conditions which was measured colorimetrically at 530 to 540 nm. The results were expressed in $\mu\text{mol/L}$.

Assessment of TAC

The assay procedure was performed according to the manufacturer's protocol (Antioxidant assay kit -709001; Cayman). Briefly, this method depends on the ability of existed antioxidants in inhibiting the oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) to $\text{ABTS}^{\bullet+}$ by a peroxidase (metmyoglobin). The amount of produced $\text{ABTS}^{\bullet+}$ was measured colorimetrically at 750 nm. The suppression of the absorbance at 750 nm, caused by antioxidant in the sample, is correlated with the antioxidant concentration. Trolox (a water-soluble tocopherol analog) was used as a standard chemical for the measurement of antioxidant levels in the samples. The results were expressed in mmol/L .

Statistical Analysis

All the data were expressed in mean (standard deviation) and analyzed using the Statistical Package for Social Sciences (SPSS version 16). Differences between groups for categorical variables were analyzed by χ^2 or Fisher exact tests as appropriate. In the case of continuous variables, Kolmogorov-Smirnov test was used to determine the use of parametric or nonparametric test. One-way analysis of variance test was applied for comparing 3 groups' differences. A 2-tailed P value of $<.05$ was considered to be statistically significant.

Results

Demographic Information and Clinical Characteristics of Patients

A total of 60 individuals (25 females and 35 males) were included in this study with the mean age of 57.9 (16.8) years. Demographic information, as well as respiratory state, SAPS score, and mortality rate of participants are summarized in Table 1.

Comparison of TAC Values Before and After the Intervention

The TAC values were assessed before and after the intervention in 3 aforementioned groups. The difference between TAC levels (mmol/L) in placebo 0.60 (0.10), intermittent 0.60 (0.06), and continuous infusion 0.63 (0.04) groups was not statistically significant (P value = .298). However, there was a considerable difference between TAC levels (mmol/L) in placebo 0.61 (0.10), intermittent 0.68 (0.06), and continuous infusion 0.69 (0.06) groups after NAC administration (P value = .007; Figure 2).

The Post hoc analysis (Scheffe) revealed that NAC considerably increased the TAC levels in both intermittent (P value = .036) and continuous (P value = .015) infusion groups when compared to the placebo. However, the difference in TAC levels between the intermittent and the continuous groups did not reach statistical significance (P value = .942).

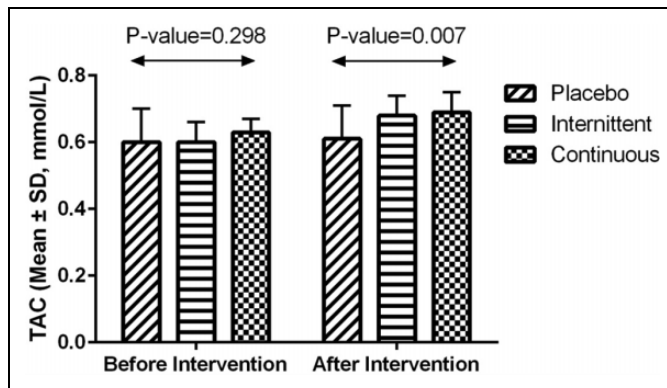


Figure 2. Comparison of TAC values difference before and after the intervention in placebo, intermittent, and continuous infusion groups. Mean (SD), $n = 20$ patients per group. TAC indicates total antioxidant capacity; SD, standard deviation.

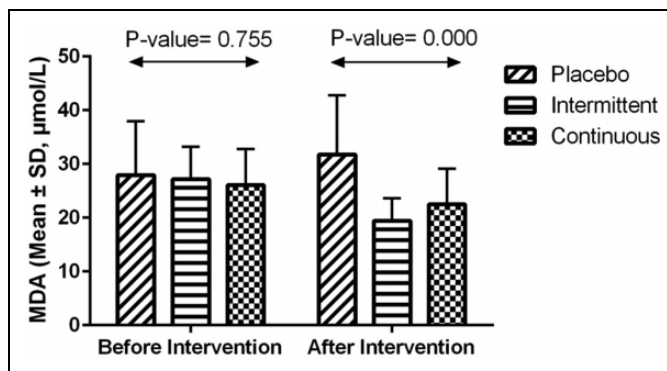


Figure 3. Comparison of MDA values difference before and after the intervention in placebo, intermittent, and continuous infusion groups. Mean (SD), $n = 20$ patients per group. MDA indicates malondialdehyde; SD, standard deviation.

Comparison of MDA Values Before and After the Intervention

Similarly, the MDA levels were measured before and after the intervention in the 3 studied groups. As it is shown in Figure 3, the difference between MDA levels ($\mu\text{mol/L}$) in the placebo 27.93 (10.06), intermittent 27.17 (6.04), and continuous infusion 26.08 (6.72) groups was not significant (P value = .755). However, there was a statistically significant difference between MDA values ($\mu\text{mol/L}$) in the placebo 31.76 (11.06), intermittent 19.45 (4.18), and continuous infusion 22.47 (6.68) groups after NAC administration (P value = .000).

The Post hoc analysis (Scheffe) showed that MDA levels were significantly lower in both intermittent (P value = 0.001) and continuous (P value = 0.002) infusion groups than that of the placebo. However, the difference in MDA levels between the intermittent and the continuous groups did not reach statistical significance (P value = .481).

Discussion

In this prospective, randomized clinical trial, there was a significant difference favoring both continuous and intermittent

infusion groups with regard to TAC and MDA levels when compared to the placebo group. However, the difference in redox status between 2 methods of NAC infusion (continuous and intermittent) did not reach statistical significance.

Sepsis with the mortality rate of 30% to 50% remains a formidable clinical challenge.³ Patients with sepsis present uncontrolled immune responses as well as relative oxidative stress.^{4,5} Moreover, it is believed that excessive generation of free radicals has a central role in the inflammatory process, thus combating the oxidative stress in initial stages of the disease is of importance.⁸ Accordingly, it has been suggested that administering exogenous antioxidant agents, namely, NAC, may be of benefit in preventing the occurrence of sepsis-induced damages.^{6,7,9}

N-acetyl cysteine is an acetylated variant of L-cysteine which presents its antioxidant activity through boosting glutathione levels. The protective functions of cells against oxidative stress are highly dependent on the thiol redox state, thus the availability of cysteine, the substrate for glutathione resynthesis, in the blood is of great importance.^{17,18} Among the agents used for maintaining the cysteine pool, NAC has been widely addressed as a safe and efficient one.¹⁰⁻¹² Furthermore, NAC has a second benefit of diminishing inflammatory cytokines.^{13,14} With regard to the role of this compound in replenishing depleted glutathione stores and promoting cellular redox state, many studies explored its effect on redox-altered diseases such as sepsis.^{10,12} Studies have shown that patients with sepsis present a depletion in the endogenous antioxidant glutathione resulting in oxidative stress¹⁹; thus, administering NAC could assist in maintaining the redox state.^{20,21} Furthermore, it is shown that NAC exerts vasodilatory functions that could show benefit in microcirculation in sepsis.⁷ Moreover, Paterson et al conducted a pilot study and showed that NAC could reduce the inflammatory responses in sepsis condition through diminishing nuclear factor- κB activation.²² Coherently, Spapen et al found that NAC could play a lung protective role by decreasing interleukin 8 as an important mediator in septic lung injury.¹⁵ It was observed that oxygenation and lung compliance improved following 4-hour infusion of NAC in patients with early diagnosed septic shock. Furthermore, the NAC-treated group had a shorter stay in the ICU in comparison to the controls.¹⁵ The NAC protective effect in sepsis was further confirmed by Ritter et al who demonstrated that the combination of NAC and deferoxamine could reduce the oxidative stress, mitochondrial dysfunction, and systemic inflammation, thereby diminishing the consequences of septic shock in a murine model of polymicrobial sepsis induced by cecal ligation and puncture.²³ Consistent with these results, Ortolani et al showed that patients with septic shock who received 70 mg/kg/d of intravenous glutathione and 75 mg/kg/d of intravenous NAC represented a remarkable decrease in peroxidative stress when compared to controls.²⁰ However, at the same time, several other contradictory findings have been published regarding some drawbacks of its clinical utility in sepsis therapy. For instance, no positive effect for NAC in sepsis was indicated by Molnar et al who found that patients with severe sepsis who received initial

intravenous bolus of NAC (150 mg/kg over 5 minutes) followed by a continuous intravenous infusion of 12.5 mg/kg per hour for 6 hours did not show any statistical significant difference in the outcomes, cytokine levels, or gastric intramucosal pH when compared to the controls.¹⁶ These results are in line with the findings reported by Szakmany et al.²⁴ It has been suggested that these contradictory data could be partly due to the dose response as well as administration timing effects of NAC. This fact necessitates researchers to pay strict attention on dosing and timing of NAC infusion in order to draw a firm conclusion from the observation.²⁵ Hence, we conducted the present study to assess the effect of NAC on the redox status of the septic patients and compare its efficacy in intermittent and continuous infusion regimens with the hope of developing the infusion regimen and optimizing the timing. Since NAC infusion for more than 24 hours has been shown to be correlated with worsening of organ failure,^{16,25,26} we administered NAC for 24 hours. The obtained results demonstrated a significant increase in TAC levels in both continuous (P value = .015) and intermittent (P value = .036) infusion groups when compared to the controls. Similarly, with regard to MDA levels, a remarkable decrease was observed in continuous (P value = .002) and intermittent (P value = .001) infusion groups in comparison with the placebo. Coherently, Galley et al who investigated the effect of intravenous antioxidants including NAC, ascorbic acid, and α -tocopherol on patients with septic shock suggested that utilizing antioxidant as an adjunct to conventional approaches could assist in the management of the disease.²⁷

However, in our study, the difference in redox status (TAC and MDA) between the 2 methods of NAC infusion (continuous and intermittent) did not reach statistical significance. To the best of our knowledge, there is no published study evaluating the efficacy of intermittent versus continuous infusion of NAC in sepsis to compare our results with.

In conclusion, our data suggest that NAC treatment in patients with sepsis could have protective effects, with no significant difference between intermittent and continuous infusion methods, and is consistent with the hypothesis that antioxidant therapy could be of importance in sepsis. However, it is well understood that antioxidant treatment will not be a definite and efficient therapy alone, since sepsis cannot be simply reduced to an altered redox pathology. Since patients with sepsis present depletion in the endogenous antioxidant glutathione, our results in terms of effectiveness of NAC suggest that use of NAC in sepsis can be considered as an adjunct to conventional approaches that hopefully could assist in the management of the disease. However, further research is needed to confirm our results.

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Declaration of Conflicting Interests

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