Impact of *N*-acetyl-L-cysteine on SARS-CoV-2 pneumonia and its sequelae: results from a large cohort study

Received: 6 Sept 2021
Accepted: 17 Nov 2021

To the Editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may cause pneumonia and acute respiratory distress syndrome (ARDS), whose pathogenesis has been partially related to an increased systemic inflammatory response with great production of pro-inflammatory cytokines causing a "cytokine storm" and an oxidative stress imbalance [1].

N-acetyl-L-cysteine (NAC) is a precursor of reduced glutathione [2] that has antioxidant, anti-inflammatory and immunomodulating properties that may prove beneficial in modulating the excessive inflammatory activation during coronavirus disease (COVID-19) [3]. Furthermore, NAC has been extensively used as a mucolytic agent to improve airway clearance in chronic respiratory diseases.

During the COVID-19 pandemic research hypotheses on the role of NAC have been formulated and randomised control trials (RCT) are ongoing; however, to date only a few case reports have been published [3, 4].

The aim of our study is to evaluate the impact of NAC administered during hospitalisation for SARS-CoV-2 pneumonia on short-term and long-term outcomes. As short-term outcomes we considered in-hospital mortality, intensive care unit (ICU) admission, length of ICU stay and length of hospital stay (LOS) in patients discharged alive; as long-term outcomes we included diffusion capacity of the lung for carbon monoxide (D_{LCO}) impairment, chest radiography alterations, reduced distance walked in the 6-min walk test (6MWT) and dyspnoea score (modified Medical Research Council (mMRC) scale) at 6 months follow-up in a subset of patients included in a follow-up study. Furthermore, we will also evaluate the impact of NAC on the development of atelectasis during hospitalisation, a possible complication of SARS-CoV-2 pneumonia.

We performed a retrospective monocentric study on 1083 consecutive adult patients hospitalised for SARS-CoV-2 pneumonia at the San Gerardo Hospital, Monza, Italy, between February 2020 and April 2021. Given that the aim was to evaluate the impact of a least 5 days of NAC administration, patients were excluded if they died or were discharged within 5 days from admission (n=177) to avoid immortal time bias. NAC was introduced, as per institutional protocol, on admission and administered at a dosage of 300 mg intravenously three times daily, switched to 600 mg per os twice daily once the patient reached clinical stability and continued until discharge. The study (STORM) was approved by the national Institutional Review Board (Spallanzani Hospital), and registered at ClinicalTrials.gov with identifier NCT04424992.

As part of a multi-centre prospective study to evaluate pulmonary sequelae caused by SARS-CoV-2 pneumonia [5] (ClinicalTrials.gov identifier: NCT04435327), we also had available follow-up data on 102 patients from the original cohort alive at discharge. The follow-up consisted of a pneumological visit at 6 months including complete pulmonary function tests and D_{LCO}, 6MWT, mMRC scale and chest radiography.

Shareable abstract (@ERSpublications)
Patients receiving *N*-acetyl-L-cysteine (NAC) during hospitalisation for #SARSCoV2 pneumonia and discharged alive present a significantly shorter length of hospital stay compared to those who did not receive NAC https://bit.ly/3l1QsVo

Cite this article as: Faverio P, Rebora P, Rossi E, *et al*. Impact of *N*-acetyl-L-cysteine on SARS-CoV-2 pneumonia and its sequelae: results from a large cohort study. *ERJ Open Res* 2022; 8: 00542-2021 [DOI: 10.1183/23120541.00542-2021].

A propensity score method was used to evaluate the impact of NAC on outcomes in the full cohort adjusting for potential confounders. We created a pseudo-population by weighting our cohort by the inverse of stabilised inverse probability of treatment weights (IPTW) computed by a multivariable logistic model on the propensity of NAC commencement with the following covariates: gender, age, days from symptoms onset to hospital admission, period of diagnosis (before/after 1 July 2020), chest radiograph (bilateral, unilateral or absence of pulmonary involvement) and ventilation support on admission, direct admission to the ICU, comorbidities (cardiovascular and cerebrovascular diseases, asthma, COPD, other pulmonary diseases, diabetes, connective tissue diseases, chronic neurological disorders, dementia and anaemia), cough and dyspnoea as symptoms and concomitant commencement of systemic steroids or remdesivir. We checked balancing among the two treatment groups after weighting by standardised mean difference and compared them by weighted two-sample rank tests [6] and a weighted logistic model. A similar approach was adopted with the subsample of subjects with a follow-up visit at 6 months.

906 patients (601 (66%) males, median age 64 years, interquartile range (IQR) 55–75 years) were included in the study, 585 (64%) received at least 5 days of NAC and 321 (36%) did not receive NAC or received less than 5 days of therapy (n=27). Demographic and clinical characteristics of the study population are summarised in table 1. Patient's characteristics were well balanced in the two groups (NAC *versus* others) after weighting with standardised mean difference always lower than 0.1.

TABLE 1 Baseline patients' characteristics and outcomes in the total original sample and in the pseudo-population weighted for the propensity score of *N*-Acetyl-L-cysteine (NAC)

	Original data			Weighted data		
	NAC commencement		SMD	NAC commencement		SMD
	No	Yes		No	Yes	
Subjects, n	321	585		329	572	
Males	192 (60)	409 (70)	0.213	209 (64)	381 (67)	0.060
Age, median (IQR)	68 (57–79)	63 (55–72)	0.232	64 (54–75)	64 (55–73)	0.003
Oxygen/ventilatory support			0.736			0.098
None	101 (31)	41 (7)		49 (15)	78 (14)	
Oxygen therapy alone	168 (52)	379 (65)		193 (59)	354 (62)	
CPAP	51 (16)	147 (25)		82 (25)	128 (22)	
ETI and IMV	1 (0)	18 (3)		5 (1)	12 (2)	
Cardiovascular diseases	187 (58)	319 (55)	0.075	178 (54)	315 (55)	0.016
Hypertension	151 (47)	275 (47)	0.001	151 (46)	270 (47)	0.021
Diabetes	69 (21)	84 (14)	0.187	53 (16)	90 (16)	0.006
Obesity	39 (23)	126 (33)	0.219	53 (26)	117 (33)	0.156
Cerebrovascular diseases	25 (8)	30 (5)	0.108	19 (6)	36 (6)	0.019
COPD	25 (8)	20 (3)	0.191	15 (5)	25 (4)	0.017
Moderate or severe chronic kidney diseases	31 (10)	41 (7)	0.096	26 (8)	43 (7)	0.022
Moderate or severe liver diseases	7 (2)	13 (2)	0.003	6 (2)	16 (3)	0.078
Chronic neurological disorders	60 (19)	52 (9)	0.287	41 (12)	74 (13)	0.014
Cancer	37 (12)	47 (8)	0.118	33 (10)	52 (9)	0.030
Immune system disorder	8 (2)	14 (2)	0.006	7 (2)	12 (2)	0.004
Treatment during hospitalisation						
Systemic steroid	76 (24)	265 (45)	0.467	122 (37)	217 (38)	0.016
Remdesivir	13 (4)	68 (12)	0.285	34 (10)	52 (9)	0.035
Outcomes						
ICU admission	20 (7)	134 (23)		46 (15)	107 (19)	0.360[#]
Days to ICU, median (IQR)	4 (1–6)	2 (0–5)		2 (0–4)	2 (0–5)	0.972[#]
Days spent in ICU, median (IQR)	9 (4–19)	14 (9–24)		20 (8–32)	14 (9–23)	0.191[#]
Hospital discharge						0.523[#]
Death	42 (13)	91 (16)		44 (13)	91 (16)	
Discharged at home	229 (71)	396 (68)		224 (68)	392 (68)	
Transferred to other facility	50 (16)	98 (17)		61 (19)	89 (16)	
Length of hospital stay in subjects discharged at home, days, median (IQR)	16 (11–25)	16 (10–25)		17 (12–30)	15 (10–24)	0.013[#]
Atelectasis during hospital stay	3 (1)	14 (2)		7 (2)	11 (2)	0.913[#]

Data are presented as n (%), unless otherwise stated. ICU: intensive care unit; IQR: interquartile range; SMD: standardised mean difference; CPAP: continuous positive airway pressure; ETI: endotracheal intubation; IMV: invasive mechanical ventilation. [#]: p-values are listed for outcomes data.

In regards to the main outcomes, 133 patients died during hospital stay (91 in the NAC group and 42 in the control group), odds ratio (OR) of mortality from the IPTW weighted logistic regression was 1.22 (95% CI 0.83–1.81, p=0.3) for the NAC group *versus* the control group. 154 patients were admitted to the ICU with a median (IQR) of 2 (0–5) days after hospital admission and with a median (IQR) time spent in the ICU of 14 (8–24) days. After adjusting by the IPTW, no differences were observed between the two groups with regards to ICU admission and length of ICU stay. LOS in patients discharged at home was lower in NAC patients (weighted median (IQR) 15 (10–24) days) compared to those who did not receive NAC (weighted median (IQR) 17 (12–30) days, p=0.013) (table 1). Occurrence of atelectasis was not different among the two groups (2% in both groups, p=0.913) (table 1).

When considering the 102 patients (78% males, median (IQR) age 59 (53–63) years) who were followed up at 6 months with a pneumological visit, no differences were observed in regards to D_{LCO} impairment (weighted percentage 24% *versus* 19%, OR 1.35, 95% CI 0.50–3.92), chest radiograph abnormalities (weighted percentage 18% *versus* 14%, OR 1.04, 95% CI 0.88–1.22) and distance walked at 6MWT (weighted median (IQR) 482 (424–540) m *versus* 480 (432–541) m, p=0.909) between patients who received NAC (n=64) and those who did not (n=38), respectively. mMRC was not statistically different between the two groups (p=0.281), although the weighted percentage of mMRC score ⩾2 (walks slower than people of the same age because of dyspnoea) was 11% in patients who received NAC and 28% in others.

Despite the preliminary evidence of a few studies, to date, there are no definitive data on the efficacy of NAC in preventing short- and long-term negative outcomes in patients with SARS-CoV-2 pneumonia [4, 7, 8]. Indeed, our results nicely fit with those of the only double-blind RCT available until now that showed no benefit of high dose NAC administration for 20 h in the emergency department on the development of severe acute respiratory failure requiring mechanical ventilation (MV) [7]. Nevertheless, LOS and long-term outcomes were not evaluated in this RCT. Similarly, a pilot study by Taher *et al.* [8] did not observe any benefit from NAC administered at a dose of 40 $mg \cdot kg^{-1} \cdot day^{-1}$ intravenously for 3 days in mild-to-moderate COVID-19-associated ARDS on long-term (overall mortality over 28 days) and short-term outcomes (including the proportion of patients requiring MV and changes in ARDS-severity 48 and 96 h after intervention) [8].

Among the main strengths of our study, we acknowledge the inclusion of consecutive patients from a tertiary care centre with a standardised protocol to manage COVID-19. This allowed patients to receive homogeneous treatment. The standardised protocol followed the indications from the evidence-based medicine: from March to July 2020 hydroxychloroquine, prophylactic heparin and, in cases of oxygen supplementation requirement, remdesivir were administered. Use of hydroxychloroquine was then discontinued from May 2020, due to the lack of efficacy in COVID-19 patients [9]. During the subsequent months (August 2020–April 2021) and after publication of the RECOVERY trial's preliminary data [10], corticosteroid therapy was administered to all patients requiring oxygen supplementation.

Among the study limitations the following must be acknowledged: the reasons that led physicians to administer or not administer NAC were not clear, leading to possible biases despite propensity matching.

In conclusion, our study does not suggest an impact of NAC on short- and long-term outcomes including in-hospital mortality, ICU admission, D_{LCO} impairment and chest radiography alterations at 6-month follow-up. Patients receiving NAC during hospitalisation for SARS-CoV-2 pneumonia presented a shorter LOS in comparison to those who did not receive NAC. However, results of the ongoing RCTs may shed further light on the role of NAC as an add-on therapy to the standard treatment for SARS-CoV-2 pneumonia.

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Acknowledgments: We would like to thank the STORM Steering Committee and Data Management for sharing data obtained from the COVID-STORM database.

Provenance: Submitted article, peer reviewed.

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Ethical approval: The STORM study was approved by national Institutional Review Board (Spallanzani Hospital). The SequelaeCoV study received Ethics Committee approval (ASST Monza, 3389, 21 May 2020).

Author contributions: A. Pesci and M.G. Valsecchi are the guarantors of this research. P. Faverio, P. Rebora, E. Rossi, S. Busnelli and A. Pesci were responsible for study concept and design. P. Faverio, S. del Giudice, F. Montanelli, L. Garzillo, S. Busnelli and F. Luppi contributed to patient recruitment and follow-up. All authors contributed to data acquisition. P. Faverio, P. Rebora, E. Rossi, S. Busnelli and M.G. Valsecchi performed data analysis. P. Faverio, P. Rebora, S. Busnelli and F. Luppi contributed to the drafting of this manuscript. All authors read and approved the final manuscript.

This study is registered at www.ClinicalTrials.gov with identifier number NCT04424992.

Conflict of interest: None declared.

Support statement: We acknowledge that this research was partially supported by the Italian Ministry of University and Research (MIUR) – Dept of Excellence project PREMIA (Precision Medicine Approach: Bringing Biomarker Research to Clinic).

References

1 Hu B, Huang S, Yin L. The cytokine storm and COVID-19. *J Med Virol* 2021; 93: 250–256.
2 De Flora S, Balansky R, La Maestra S. Rationale for the use of *N*-acetylcysteine in both prevention and adjuvant therapy of COVID-19, 2020. *FASEB J* 2020; 34: 13185–13193.
3 Shi Z, Puyo CA. N-acetylcysteine to combat COVID-19: an evidence review. Therapeutics and clinical risk management. *Ther Clin Risk Manag* 2020; 16: 1047–1055.
4 Ibrahim H, Perl A, Smith D, *et al.* Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous n-acetylcysteine. *Clin Immunol* 2020; 219: 108544.
5 Faverio P, Luppi F, Rebora P, *et al.* Six-month pulmonary impairment after severe COVID-19: a prospective, multicenter follow-up study. *Respiration* 2021; 100: 1078–1087.
6 Lumley T, Scott AJ. Two-sample rank tests under complex sampling. *Biometrika* 2013; 100: 831–842.
7 de Alencar JCG, de Lucena Moreira C, Müller AD, *et al.* Double-blind, randomized, placebo-controlled trial with N-acetylcysteine for treatment of severe acute respiratory syndrome caused by Coronavirus Disease 2019 (COVID-19), 2021. *Clin Infect Dis* 2021; 72: e736–e741.
8 Taher A, Lashgari M, Sedighi L, *et al.* A pilot study on intravenous *N*-acetylcysteine treatment in patients with mild-to-moderate COVID19-associated acute respiratory distress syndrome. *Pharmacol Rep* 2021: 73: 1650–1659.
9 The RECOVERY Collaborative Group, Horby P, Lim WS, *et al.* Dexamethasone in hospitalized patients with Covid-19, 2021. *N Engl J Med* 2021; 384: 693–704.
10 Elavarasi A, Prasad M, Seth T, *et al.* Chloroquine and hydroxychloroquine for the treatment of COVID-19: a systematic review and meta-analysis, 2020. *J Gen Intern Med* 2020; 35: 3308–3314.