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N-Acetylcysteine as A Potential Adjunctive Theraphy in Covid-19 Patients Associated Coagulopathy by Lowering Il-1β, D-dimer and Padua Scores Levels

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ABSTRACT

Introduction: Many unique pathologies, such as hyperinflammation and hypercoagulopathy, have been associated with Covid-19, resulting in critical illness and poor outcomes. N-Acetylcysteine (NAC), a precursor of the antioxidant glutathione, has a number of biological effects, including increasing antioxidant capacity, inhibiting viral replication, and suppressing the release of proinflammatory cytokines, such as IL-1β, which further reduces coagulopathy as measured by D-dimer and PADUA scores. The aim of this research is to analyze the effect of NAC as an adjunctive therapy on the level of IL-1β, D-dimer and PADUA scores among Covid-19 patients.

Method: A quasi-experimental, non-equivalent group, single-center study included 91 hospitalized confirmed Covid-19 patients by RT-PCR, 75 patients received standard of care plus 5000 mg/72 hours of NAC and 16 patients received standard of care without NAC. On the first and eighth days of treatment, IL-1β, D-dimer levels, and PADUA scores were measured by ELISA and quantitative-assay methods. Data analysis used were Mann-Whitney test, Wilcoxon test, and Spearman-rho correlation test.

Result: After the eighth day of therapy, the NAC group had a statistically significantly reduction in IL-1β(pg/mL) (1,48[-4,23-6,68] vs -0,43[-3,40-2,0], p=0.03) and D-dimer(µg/mL) (0,75[-5,98-6,45] vs -0,03[-8,07-1,00], p<0,001) levels than the control group, but not in PADUA scores (1,05[0-3] vs 0,94[0-1], p=0,458). In a subanalysis of NAC's group, IL-1β was found to be quite strongly correlated with D-dimer (r=0,508, p<0,001).

Conclusion: Our findings show that supplementary NAC reduces hyperinflammation and hypercoagulopathy in Covid-19 patients by suppressing IL-1β and lowering D-dimer, and thus could be used as a supplement to Covid-19 therapy.

Keywords: COVID-19, N-acetylcysteine, IL-1β, coagulopathy, D-dimer

1. INTRODUCTION

COVID-19, a global pandemic since March 2020, is linked to coagulation diseases, such as disseminated

intravascular coagulation and hypercoagulable states, which are becoming more common and are linked to poor outcomes and even high fatality rates. Corona virus infection can activate multiple systemic coagulation factors and inflammatory responses, according to various suggested pathways of immunopathogenesis. Increased production of proinflammatory cytokines occurs as a result of the host's inflammatory response, leading to coagulation factor activation and consumptive coagulopathy[1,2,3,4].

Numerous studies demonstrate the potential of several novel anticoagulants with a cellular mechanism to inhibit the formation of these NETs, despite the fact that many experts urge prophylactic anticoagulant medication[3]. After being triggered by an infection, neutrophils rush to the site of inflammation in the body, where they manufacture and release more potent cytokines, such as neutrophil elastase (NE) and reactive oxygen species (ROS), as well as DNA, which makes neutrophil even more powerful[5].In human epithelial cells, N-acetylcysteine (NAC), a precursor to glutathione, has been shown to suppress NF-kB (which is known to create more potent pro-inflammatory cytokines) and virus influenza (strain H5N1, Vietnam / VN1203) reproduction[6].

IL-1$\boldsymbol{\beta}$ is thought to cause ceramide production and NETosis in aortic infiltrating neutrophils, which contributes to the formation of aortic aneurysms. Other research has mentioned the role of IL-1$\boldsymbol{\beta}$, which is triggered and promotes the inflammasome, has a role in neutrophil, monocyte, and platelet recruitment, and stimulates VTE/Venous Thrombo Embolism. Through increased G-CSF production and participation in NET formation, IL-1$\boldsymbol{\beta}$ may be a target that drives cancer-associated thrombosis events[7,8,9] . The aim of this research is to analyze the effect of NAC as adjunctive therapy on the level of IL-1$\boldsymbol{\beta}$, D-dimer and PADUA *scores* among Covid-19 patients.

2. MATERIALS AND METHODS

The study's participants were confirmed (+) patients, both non-intensive and intensive room patients, and the method utilized in this study was Quasi Experimental Non-equivalent Control Group Design. This study was approved by an Independent Ethics Committee. All subjects signed a written informed consent form before enrollment.

75 patients as a subject group were given standard of care and additional medication NAC 5000 mg/72 hours as an antioxidant dosage (dose > 1200 mg/day) after receiving their RT-PCR results and meeting the inclusion criteria. 16 patients as in the control group received standard of care without NAC but used the same technique or flow as the treatment group. Then, on the first and seventh days after treatment, pre- and posttreatment evaluations of IL-1$\boldsymbol{\beta}$, D-dimer and PADUA *scores* were conducted (figure 1).

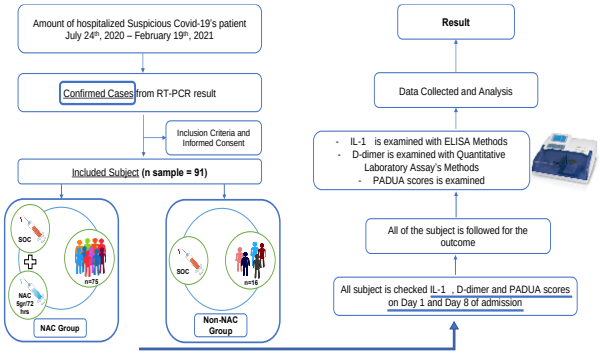


Figure 1. Research Methods

3. RESULTS

Variable and Cathegory	NAC Group	Non-NAC Group	*p. Sig*
Age (Median, (Min-Max))	56.00, (24 – 76)	52.50, (25 – 69)	*p=0.577*
Gender			
- Male	44 (58.7%)	12 (75%)	*p=0.225*
- Female	31 (41.2%)	4 (25%)	
Severity			
- Moderate	33 (44.0%)	3 (18,8%)	*p=0.526*
- Severe	21 (28.0%)	11 (68,8%)	
- Critically Ill	21 (28.0%)	2 (12,5%)	
Admission's Complaint			
- Shortness of Breath	61 (81.3%)	16 (100%)	*p=0.062*
- Cough	60 (80%)	16 (100%)	*p=0.052*
- Fever	53 (70.6%)	16 (100%)	*p=0.013*
- Anosmia/Augesia	15 (20%)	10 (62.5%)	*p=0.001*
- GIT disturbances	42 (56%)	10 (62.5%)	*p=0.635*
Recent Smoker or History of Smoking			
- Yes	34 (46.67%)	10 (62,5%)	*p=0.091*
- No	41 (54.67%)	6 (37,5%)	
Comorbidities			
- Yes	34 (46.67%)	7 (43.75%)	*p=0.909*
- No	41 (54.67%)	9 (56.25%)	
Outcome			
- Recovery	64 (85,3%)	15 (93,8%)	*p=0.369*
- Death	11 (14,7%)	1 (6,3%)	
Length of Stay	13,56 ± 4,226	13,87 ± 6,649	*p=0.001*

Figure 2. Demographic Table of Research Subject

The median sample age in the two groups, 56 vs 52.5 years, was not statistically different, according to the demographic table (figure 2). Similarly, in both categories, there are more male samples than female samples. The severity of the patients admitted to the two groups did not differ considerably, with severe and critical degrees being the most common when admitted to the ER, and complaints of shortness of breath, cough, and fever being the most common when admitted to the hospital. With p-Sig = 0.369, there was no significant difference in the treatment outcome between the groups who received NAC and those who did not get NAC. However, there was a significant difference in the length of hospitalization between the two groups, with the length of stay in the NAC group being shorter than the length of stay in the non-NAC group (13.56 ± 4.226 days vs. 13.87 6.649 days, p-Sig = 0.001).

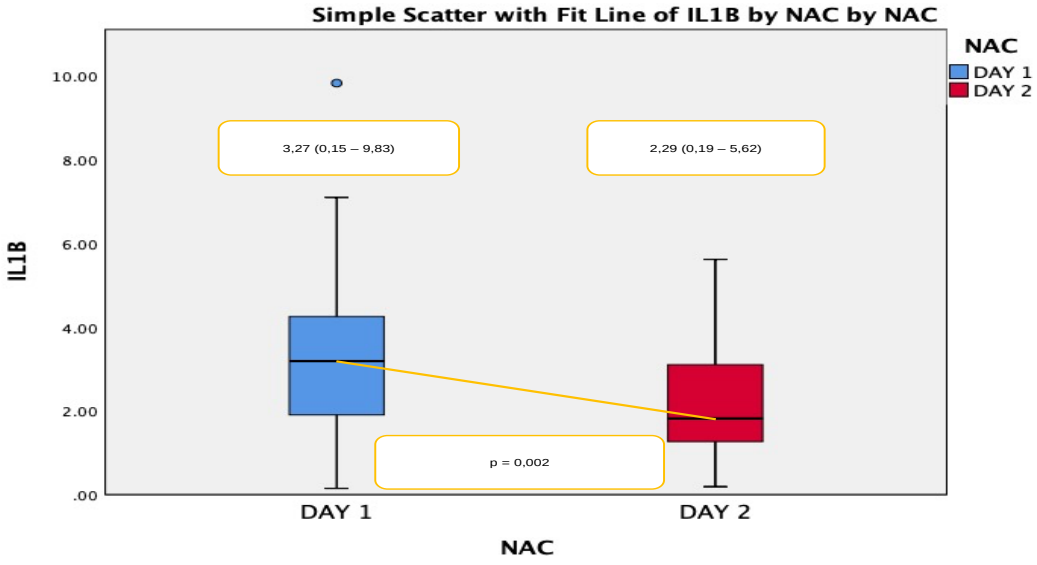


Figure 3. Comparison of changes in IL-1β in the NAC groups on day 1 and 8

Figure 3 shows that on day 8, when compared to H-1, there was a substantial drop in IL-1β (3.27 (0.15 – 9.83) to 2.29 (0.19 – 5.62), p=0.002). The results were not significant in the group that did not get NAC (figure 4).

On day 8, the concentration levels of D-dimer in the NAC group were lower (2.19 (0.38 – 8.37) than in the control group (1.15 (0.11– 6.43), p<0.001) (figures 5 and 6).

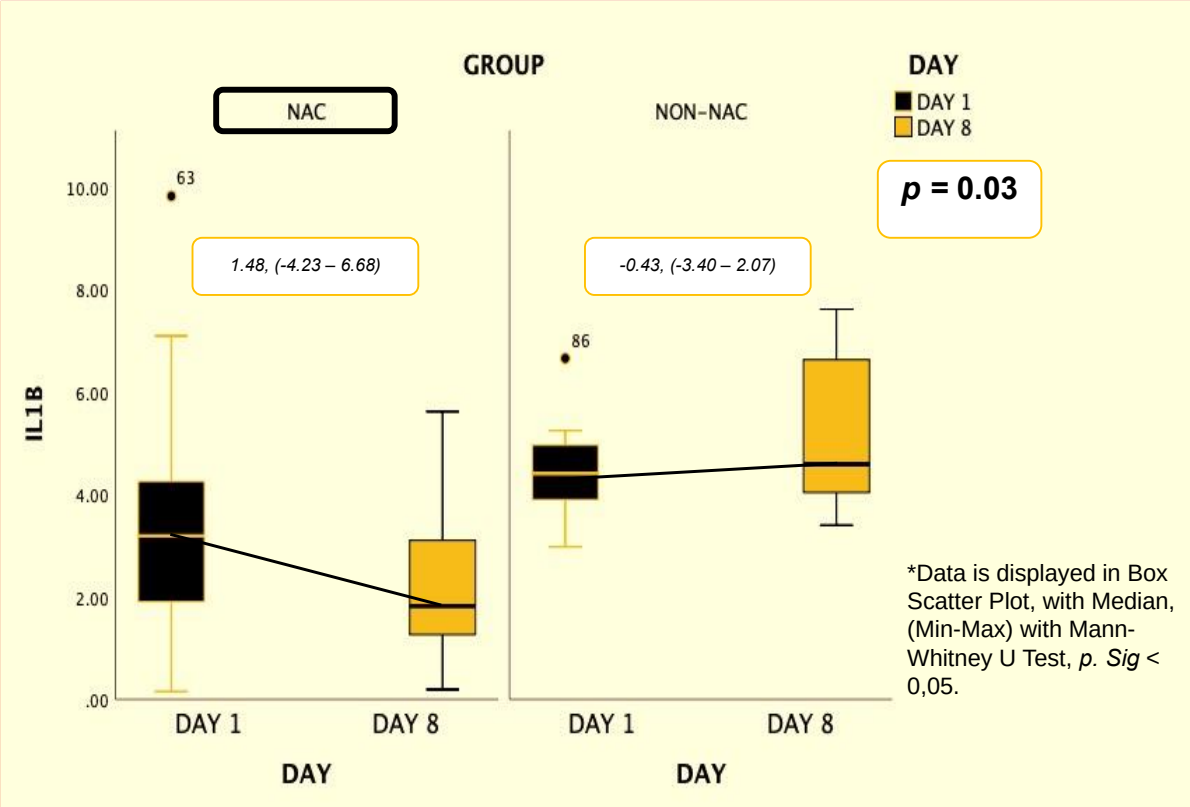


Figure 4. Comparison of changes in IL-1β between the NAC and Control groups on day 1 and 8

This study also looked at changes in PADUA scores and their relationship to each variable (figure 7 and figure 8), finding that while there was a decrease, there was no significant difference between the NAC and Non-NAC groups (1, 0–3) versus 1, (0–1), p = 0.458 (figure 7 and figure 8).
In our subanalysis, there is a substantial positive association between changes in IL-1β and changes in D-dimer in Covid-19 patients after adjuvant NAC medication, but no correlation between changes in IL-1β and PADUA scores or D-dimer to PADUA scores was discovered, with r=0.508, p<0,001, n=75.

4. DISCUSSION

In a meta-analysis of the impact of age, gender, comorbidities, and clinical features on COVID-19, it was discovered that older male patients (>50 years) had a higher chance of acquiring COVID-19 (OR = 2.41, p 0.00001). ; 3.36, p = 0.0002)[10].

Men have an 18% higher risk of developing severe COVID-19 disease than women[11]. According to a meta-analysis study conducted in China, the majority of COVID-19 cases received were severe and critical (17.84 percent and 4.9 percent respectively). Furthermore, most present with cough, shortness of breath, exhaustion, fever, and gastrointestinal symptoms, and they tend to have a more severe illness[12].

Another meta-analysis study found that fever, 60.70 percent cough, 33.21 percent weariness or myalgia, 31.30 percent dyspnea, and 10.65 percent diarrhea were the most common reasons for hospitalization in

COVID-19 patients[13]. Similarly, there was no significant difference in smoking history between the two groups (p-Sig = 0.091). With p Sig = 0.909, the majority of people had comorbidities.

Smoking history and active smoking were both linked to severe COVID-19 in one study (OR = 1.51; 95 percent CI: 1.12–2, 05). COVID-19 was found in 10.7% (978/9067) of nonsmokers and 21.2 percent (65/305) of active smokers[14]. A systematic review found that NAC reduces the length of stay in the intensive care unit but does not reduce overall mortality in ARDS patients (8 trials with a total of 289 patients)[15].

NAC at high concentrations (from 300 M to 10 mM) was shown to strongly reduce the release of IL-1$\boldsymbol{\beta}$ (33.62 2.10 percent), IL-8 (68.11 17.34 percent), and TNF-A (62.82 10.34 percent) generated by lipopolysaccharide (LPS) in an ex vivo model of COPD exacerbation in multiple prior investigations[16]. Lower GSH or glutathione levels, greater ROS levels, and a higher redox state (ROS/GSH ratio) are all linked to COVID-19 instances[17,18].

Increased plasma concentrations of inflammatory markers like CRP and ferritin, as well as proinflammatory cytokines like TNF—alpha, IL-1$\boldsymbol{\beta}$, IL-6, and IL-8, and chemokines like MCP1, as well as a higher neutrophil/lymphocyte ratio, have been linked to an increased risk of SARS-CoV2 infection and death. Although lung and other tissue damage in SARS-CoV2 infection may be caused by a multifactorial mechanism, current research suggests that reactive oxygen species (ROS) may play a key role in the onset and advancement of this inflammatory process. Through NF-kB and thioredoxin, which interact and limit protein activation, ROS activate the NLRP3 inflammasome. Furthermore, NF-kB inhibits the expression of IL-18 and IL-1$\boldsymbol{\beta}$, boosting the NLRP3 inflammasome even further[19].

Another study looked at the effects of NAC treatment up to days 7 and 14 (p.001 at day 7 and day 14), finding enhanced oxygenation and lower levels of leukocyte CRP, and D-dimer with time, demonstrating the mechanism of anti-inflammatory NAC[20].

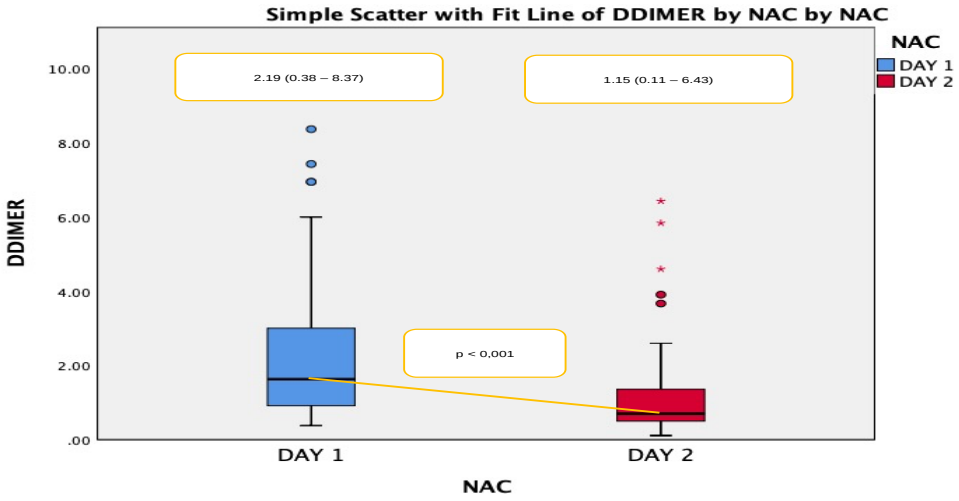


Figure 5. Comparison of changes in D-dimer in the NAC groups on day 1 and 8

Several in vitro and in vivo investigations have examined the possible mechanism of action of NAC. NAC, in addition to being a glutathione precursor, has been linked to a number ofmechanisms, including

downregulating the NLRP3 or inflammasome's mRNA expression, which reduces the expression of proinflammatory cytokines and their release from activated mononuclear phagocytes, and thus inhibiting the release of IL-1β, IL-8, and TNF-, which is linked to lower levels of IL-1β, IL-8, and TNF- [20].

There haven't been many studies that link IL-1β and D-dimer to PADUA scores, but one study found that a combination of CURB-65 scores 3 to 5, PADUA predictive score 4, and D-dimer >1.0 g/mL had a sensitivity of 88.52 percent and a specificity of 61.43 percent for DVT screening in COVID-19 patients[21].

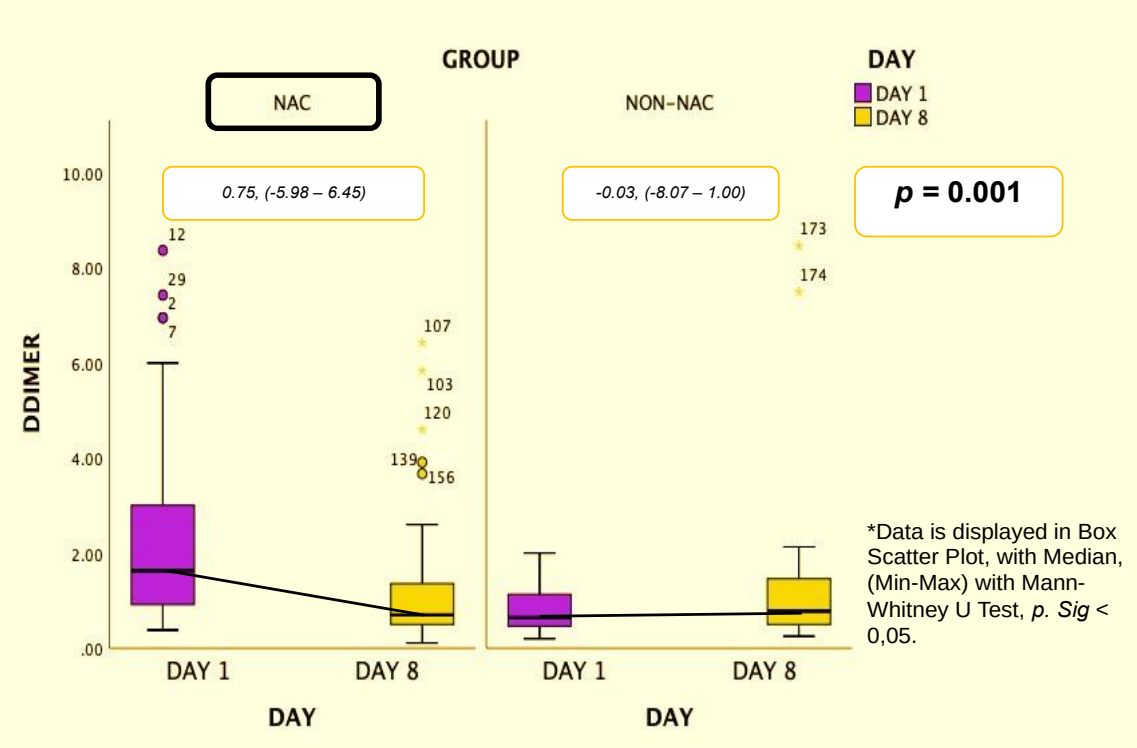


Figure 6. Comparison of changes in D-dimer between the NAC and Control groups on day 1 and 8

High PADUA scores [odds ratio (OR): 7.35, 95 percent confidence interval (CI): 3.08-16.01], increased IL-6 (OR: 11.79, 95 percent CI): 5.45-26.20), and increased D-dimer (OR: 4.65, 95 percent CI: 1.15-12.15) were found to be independent risk factors for in-hospital mortality in a multivariate logistic regression study[21].

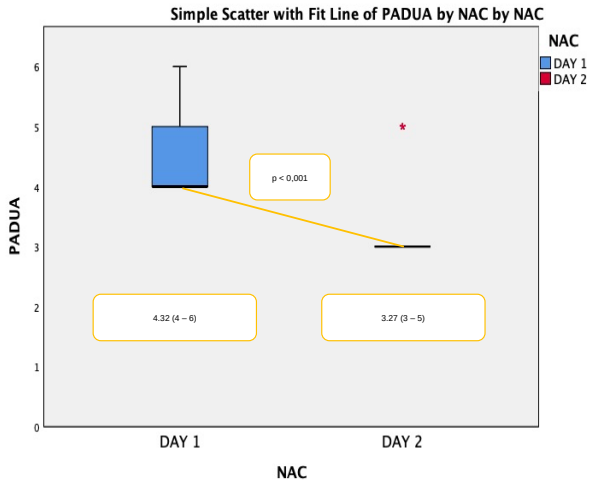


Figure 7. Comparison of changes in PADUA scores in the NAC groups on day 1 and 8

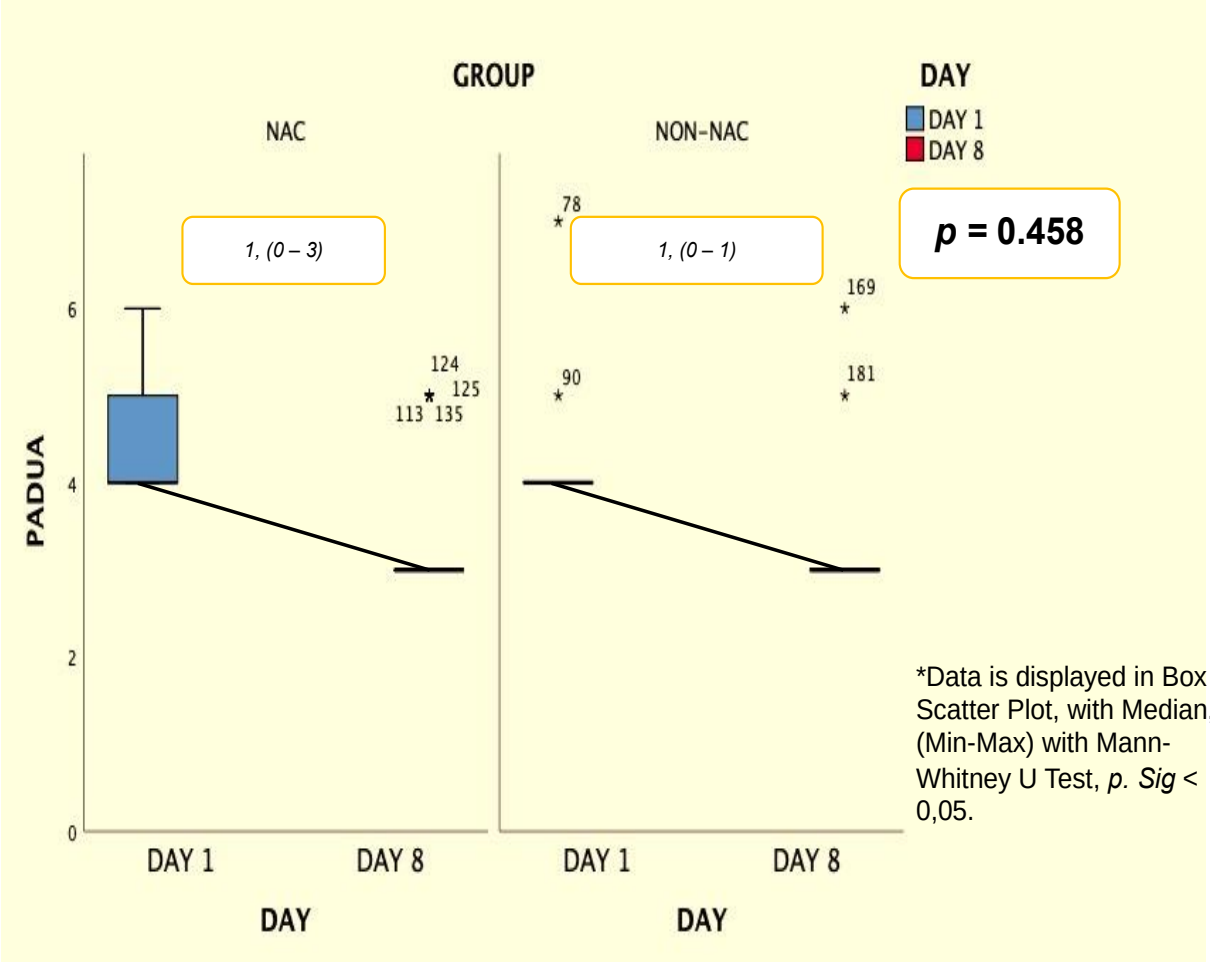
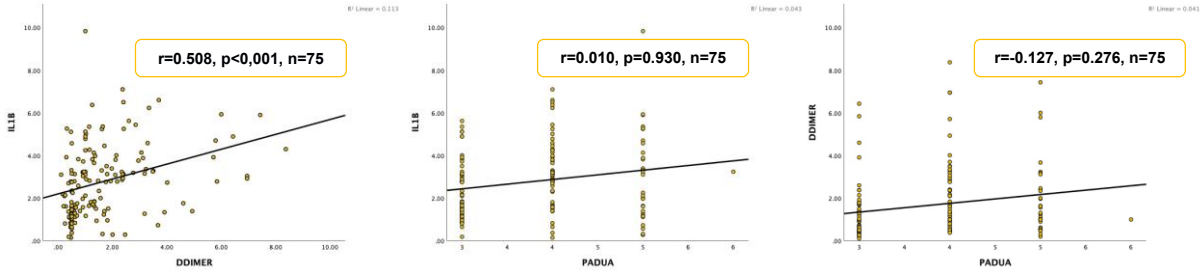


Figure 8. Comparison of changes in PADUA scores between the NAC and Control groups on day 1 and 8

According to a 2012 study, neutrophils can produce enormous amounts of tissue factor/TF in the form of NETs. They also proved that NET-associated TF is produced by neutrophils, and that the thrombin results can activate platelets. The same scientists discovered that proinflammatory cytokines (IL-1***β*** and TNF-) must be used to prime neutrophils for TF mRNA translation[22,23]. According to the study, they had higher levels of NETosis as measured by plasma MPO-DNA complex and plasma tissue factor complex TF-DNA. After analysis, it was discovered that circulating IL-1***β*** levels were associated with an patients with STEMI and high CRP levels increase in circulating myeloperoxidase (MPO)-DNA and TF-DNA complexes, as well as CRP (n = 66). **p<0.01)**p<0.01)**p<0.01)**p<0.01)** [23].



*Data is displayed with Spearman's rho' Test, *p. Sig* < 0,05.

Figure 9. Correlation between IL-1*β*, D-dimer and PADUA scores in NAC group

Increased plasma DNA levels (Plasma DNA and nucleosomes assessed as NETosis-specific biomarkers) were linked to cancer-related stroke and increased D-dimer levels >2 g/mL in a multiple regression analysis research (r=0.492, p0.001)[24].
A favorable connection between higher plasma NETosis biomarker levels and D-dimer (r=0.5, p0.001) has also been reported in COVID-19 patients in several investigations[25].

5. CONCLUSION

In summary, supplemental 5 gr/72 hours of NAC lowers hyperinflammation and hypercoagulopathy in Covid-19 patients by suppressing IL-1$\boldsymbol{\beta}$ and lowering D-dimer but not with PADUA scores, and so could be utilized as a complement to Covid-19 therapy, according to our findings.

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DISCLOSURES

LTH, SDP, SD, ASL, and HAR declare that they have no conflict of interest. This study was carried out in accordance with the Declaration of Helsinki, the International Council for Harmonization consolidated guideline for Good Clinical Practice, and local regulations and were approved by an Independent Ethics Committee. All subjects signed a written informed consent form before enrollment.

AUTHOR CONTRIBUTIONS

LTH, SDP, SD, ASL, and HAR contributed to the design of the study and protocols. LTH conducted the study and directed the studies at hospital. LTH and HAR analyzed/ interpreted the data, SDP, SD, and HAR critically revised the manuscript, agreed on the content, and approved the final version for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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