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Cohort Study

An observational cohort study to assess N-acetylglucosamine for COVID-19 treatment in the inpatient setting

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) has affected millions globally, with a continued need for effective treatments. N-acetylglucosamine has anti-inflammatory activities and modulates immune response. This study evaluated whether N-acetylglucosamine administered orally improves clinical outcomes for patients admitted to the hospital due to COVID-19.

Materials and methods: This single-center, prospective, observational cohort study used a retrospective control group for comparison. Multivariate analyses evaluated whether N-acetylglucosamine was an independent predictor of primary outcomes (rate of intubation, hospital length-of-stay, and mortality) and select secondary outcomes (intensive care unit [ICU] admission, ICU length-of-stay, supplemental oxygen use duration, hospice initiation, and poor clinical outcome [defined as combined hospice initiation/death]).

Results: Of the 50 patients enrolled in the N-acetylglucosamine treatment group, 48 patients had follow-up data (50.0% [24/48] male; median age 63 years, range: 29–88). Multivariate analysis showed the treatment group had improved hospital length-of-stay (β: 4.27 [95% confidence interval (CI) −5.67; −2.85], $p < 0.001$), ICU admission (odds ratio [OR] 0.32 [95% CI 0.10; 0.96], $p = 0.049$), and poor clinical outcome (OR 0.30 [95% CI 0.09; 0.86], $p = 0.034$). Mortality was significantly lower for treatment versus control on univariate analysis (12.5% vs. 28.0%, respectively; $p = 0.039$) and approached significance on multivariate analysis ($p = 0.081$).

Conclusions: N-acetylglucosamine administration was associated with reduced hospital length-of-stay, ICU admission rates, and death/hospice rates in adults with COVID-19 compared to those who received standard care alone. An upcoming trial will further investigate N-acetylglucosamine's effects.

Trial Registration: NCT04706416.

1. Introduction

As of April 2021, there have been over 141 million cases of coronavirus disease 2019 (COVID-19) globally, with more than 31 million of those cases and 567,000 deaths occurring in the United States [1]. A number of therapies have been used and investigated for COVID-19 treatment, including corticosteroids [2,3], antiviral agents [4–6], immunomodulatory drugs [7], serotherapy and convalescent plasma [8], anticoagulants [9], and inflammatory inhibitors [10,11]. Of these, the United States Food and Drug Administration has only given emergency use authorization to remdesivir and tocilizumab for treatment against COVID-19 [12,13] and prognosis for the disease remains poor [14]. Therefore, it is important to continue searching for treatments that can slow or reverse clinical deterioration associated with COVID-19 illness.

N-acetylglucosamine (NAG) is a naturally occurring amino sugar precursor for epithelial glycosaminoglycan synthesis and has been used to reduce chronic inflammation associated with osteoarthritis [15,16]. Researchers proposed mechanisms for glucosamine-mediated inhibition of enveloped virus replication as early as the 1970's [17–19]. More recent studies describe NAG's involvement in human bronchial epithelial cell immune function via the hexosamine biosynthetic pathway [20], and multiple animal models have indicated that NAG may protect organ function in acute settings by reducing maximum inflammation [21,22]. Given the anti-inflammatory and immune activities of NAG, this study sought to analyze the effects of 700 mg NAG administered twice daily to patients with COVID-19.

The safety and rationale for using NAG on the described dosing schedule have been well established. Human clinical trials for NAG as treatment for osteoarthritis have nearly universally employed a dose of 1500 mg/day [15,23], closely aligning with the 1400 mg/day total

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Abbreviations

COVID-19	coronavirus disease 2019
LOS	length-of-stay
NAG	N-acetylglucosamine
ICU	intensive care unit

dosing in the current study. In a pilot study by Salvatore et al., NAG was administered in doses ranging from 3 to 6 g with no adverse side effects of the treatment noted [24]. NAG is safe when administered intravenously in quantities as large as 20 g in humans, with no toxicity or alteration of blood glucose concentration [16,25]; likewise, the long-term safety of NAG has been endorsed due to the relative absence of treatment-related adverse effects in the literature [26].

While the safety of NAG is clear, it is unclear what effects its administration will have on various clinical outcomes. Therefore, this study investigated the effects of NAG administration on rates of intubation, hospital length of stay, and mortality in COVID-19 patients.

2. Methods

2.1. Study design

This work has been reported in line with STROCSS criteria [27]. This was a single-center, observational study carried out in adult patients presenting to the emergency department of Valley Baptist Medical Center (Harlingen, TX, USA) with shortness of breath. The research protocol was reviewed and received approval from the Institutional Review Board (IRB) at MetroWest Medical Center, Framingham Union Hospital, and Leonard Morse Hospital (IRB #2020-106; approved August 26, 2020). The study is registered on ClinicalTrials.gov (NCT04706416). The study period was November 14, 2020, to January 15, 2021, aiming to enroll 50 patients to receive NAG.

Consecutive patients who presented with shortness of breath were immediately tested for COVID-19 through reverse transcription polymerase chain reaction (RT-PCR) and approached for enrollment in the study. Those who provided informed consent and received a positive COVID-19 diagnoses were included in the study; those who tested negative for COVID-19 were not included. No determination was made regarding COVID-19 variants or false positive results. Study participants received 700 mg NAG orally every 12 h as first-line treatment upon admission. Patients in the treatment group also received standard of care at the discretion of the attending physician, including antibiotics, antivirals, corticosteroids, and convalescent plasma. Patients continued to receive NAG and were followed until study exit, which occurred at expiration, discharge, or 30 days.

2.2. Inclusion & exclusion criteria

Inclusion criteria, which remained unchanged for the duration of the study, stipulated that all patients had to be $\geq$ 18 years old; present with shortness of breath (since local institutional policy only admitted patients with shortness of breath); clinical diagnosis of COVID-19 by RT-PCR; hospital admittance due to COVID-19; were administered NAG orally as first-line treatment; and no intubation prior to hospitalization and enrollment in the current study. Patients were excluded if they did not meet criteria above, had an allergy to NAG or shellfish, were currently taking warfarin, or currently pregnant or lactating.

2.3. Data collection & outcomes

Upon admission, the research team recorded patient demographics, comorbidities, symptoms, disease severity (as assessed by the World Health Organization [WHO] Ordinal Scale for Clinical Improvement [28]; see Table S1), need for supplemental oxygen, and time from symptom onset until hospital arrival. The research team also collected bloodwork for the following at admission: white blood cell count (WBC), hematocrit (HCT), hemoglobin (HBG), C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), and erythrocyte sedimentation rate (ESR). During the study period, discretionary treatments and interventions were recorded daily until study exit.

The primary outcomes of interest were rate of intubation, hospital LOS, and mortality following rapid administration of 700 mg NAG for COVID-19 treatment. Secondary outcomes of interest included intensive care unit (ICU) admission, ICU LOS, supplemental oxygen duration, rate of hospice initiation, and poor clinical outcome (defined as combined death/hospice initiation).

2.4. Comparison groups & statistical analysis

Beginning on the study start date, the previous 100 COVID-19-positive consecutive patients admitted to Valley Baptist Medical Center were retrospectively identified via chart review to serve as the control/comparison arm. Data for these patients was collected before commencement of the NAG trial. Univariate analysis was performed for all primary and secondary outcomes, followed by multivariate analysis for primary outcomes and select secondary outcomes that approached significance and had sufficient frequency of occurrence to be meaningful; the main conclusions of this study are drawn from multivariate analysis. Due to its status as a pilot study and the urgency of releasing information about COVID-19, a priori sample size calculations were not performed for this study. Instead, samples were based on cost, patient availability, limited timeline, and best practices in the view of the principal investigator, with the intention of detecting potential effects or trends to be further evaluated in later studies. Since IRB did not require patient consent for de-identified data and there was sufficient data availability, a larger study arm was selected for the control arm to increase the power and sensitivity of the analysis while reducing risk of type 2 error, at no extra cost in terms of time or resources.

Continuous parameters were assessed for normality based on cross-validation using Anderson-Darling [29], D'Agostino-Pearson omnibus [30], Shapiro-Wilk [31], and Kolmogorov-Smirnov tests [32]. [32] Comparisons of normally distributed data between groups were analyzed using unpaired Student's t-tests. Effect sizes from t-tests were reported as mean differences with 95% confidence intervals (CIs) based on normal approximation. Comparisons of nonparametric data between groups were analyzed using the Mann-Whitney *U* test. Effect sizes from nonparametric tests were reported as Hodge's-Lehman differences (H-L Diff) and their respective 95% CIs. Comparisons of dichotomous data between groups were analyzed using Fisher's exact binomial test [33]. Effect sizes from Fisher's exact test were reported as odds ratios (ORs) with 95% CIs computed using the Baptista-Pike method [34].

A correlation matrix showing the strength and direction of correlation between covariates and outcomes was generated using Spearman's rank correlation test via the 'corrplot' package in R. Spearman's rank correlation was used to provide a more robust measurement of correlation in the face of high leverage outliers. Simple linear or logistic regressions were used to evaluate potential predictors of primary and secondary outcomes. Multivariate regression using best subset selection was performed based on model comparison using adjusted R^2 values (linear regressions) or adjusted pseudo-R^2 values (logistic regressions). Model predictive performance of multiple linear regression was also evaluated by root mean square error (RMSE) values and multiple logistic regressions were evaluated by area under the receiver operating characteristic curve (ROC). Comparisons of best subset selection with models including the full set of covariates considered for multivariate analysis was also performed. Best subset selection was carried out using the 'leaps' package in R. P-values $\leq$0.05 were considered significant for all analyses. All statistics were performed in RStudio (Version 1.3.959,

RStudio, PBC, Boston, MA).

3. Results

Of the 50 patients enrolled in the prospective cohort study, 48 patients had available follow-up data. The first treatment arm patient was enrolled on November 14, 2020, and recruitment was completed on December 12, 2020; the control arm patients arrived at the hospital between July 7, 2020, and November 14, 2020. The treatment group had median age of 63 years (range: 29–88) and was 50.0% (24/48) male, whereas the patients in the control arm had median age of 68 years (range: 23–95) and was 62.0% (62/100) male. The NAG group was significantly younger than the control group (H-L Diff: 5.0 [95% CI: 0.0; 10.0], p = 0.049). Time from symptom onset to admission was significantly longer in the NAG group compared to the control group (H-L Diff: 3.0 [95% CI: 6.0; −1.0], p = 0.008). Hypertension was less prevalent in the NAG group compared to the control group (OR: 0.35 [95% CI: 0.18; 0.71], p = 0.004). Antiviral administration was more frequent in the NAG group compared to the control group (OR: 2.58 [95% CI: 0.88; 7.55], p = 0.001). A full list of demographics and clinical characteristics upon admission are in Table 1. Outputs of normality tests are shown in Table S2.

3.1. Univariate analysis of outcomes

Initial analyses of all primary and secondary outcomes are presented in Table 2. The NAG group was significantly less likely to have mortality (12.5% vs. 28.0%, respectively; OR: 0.37 [95% CI 0.15; 0.91], p = 0.039) and poor clinical outcome (OR: 0.30 [95% CI 0.12; 0.80], p = 0.015) compared to the control group. There were no significant differences for intubation rates, hospital LOS, ICU admission, ICU LOS, duration of oxygen use, or hospice initiation between the NAG and control groups based on univariate analysis.

3.2. Multivariate analysis of primary outcomes

A correlation matrix showing all pairs of correlation coefficients between all pairs of covariates is presented in Fig. S1. Following best subset selection for multivariate analysis to maximize adjusted R^2 values, NAG was not shown to be a significant independent predictor of reduced intubation rate (OR: 0.68 [95% CI: 0.19; 2.30], p = 0.541) (Table 3). NAG was shown to be a significant independent predictor of reduced hospital LOS (β: 4.27 [95% CI: 5.67; −2.85], p < 0.001) (Table 4). Finally, NAG was not a significant independent predictor of reduced mortality rate (OR: 0.34 [95% CI: 0.09; 1.07], p = 0.081) (Table 5). Summaries of the model selection process for intubation, hospital LOS, and mortality are shown in Figs. S2–4, respectively.

3.3. Multivariate analysis of select secondary outcomes

Multivariate analyses were performed for two secondary outcomes of interest: poor clinical outcome and ICU admission. On multiple logistic regression, NAG was shown to be a significant independent predictor of reduced poor clinical outcome rate (OR: 0.30 [95% CI: 0.09; 0.86], p = 0.034; see Table S3) and reduced ICU admissions (OR: 0.32 [95% CI: 0.10; 0.96], p = 0.049; see Table S4). Summaries of the model selection process for poor clinical outcome and ICU admission are shown in Figs. S5 and S6, respectively.

4. Discussion

On multivariate analysis, this observational study indicated that NAG administration was a significant independent predictor of reduced rates of ICU admission, hospital LOS, and hospice initiation/death for COVID-19 patients compared to those in the control arm. NAG was not a significant independent predictor of mortality alone on multiple logistic

Table 1
Patient characteristics at baseline.

Characteristic	NAG (n = 48)	Control (n = 100)	Comparison	p-value
Age (years)	63 (51.50–72.25) [29–88]	68 (57.25–76.75) [23–95]	5.0 (0.0; 10.0)	0.049[b]
Sex (male)	24 (50%)	62 (62%)	0.61 (0.30; 1.23)	0.213
Race	–	–	1.17 (0.38; 3.16)	>0.999
Hispanic	43 (89.6%)	88 (88%)	–	–
White	5 (10.4%)	12 (12%)	–	–
Time from symptom onset (days)	11.5 (4.75–11) [1.42–32]	7 (4–15.75) [1–59]	−3.0 (−6.0; −1.0)	0.008[b]
Disease severity[c]	4 (4–5) [3–5]	4 (4–5) [3–7]	0.0 (0.0; 0.0)	0.156
Supplemental oxygen	46 (91.7%)	86 (86%)	1.79 (0.56; 5.22)	0.425
Bloodwork				
WBC	8.65 (6.05–11.73) [1.1–20]	10.25 (6.725–13.50) [3.3–29.3]	1.4 (−0.1; 3.1)	0.070[b]
HCT	39.71 ± 6.02	37.97 ± 7.93	−1.74 (−4.29; 0.82)	0.181
HGB	12.69 ± 1.91	12.10 ± 2.61	−0.59 (−1.45; 0.27)	0.175
Comorbidities				
Asthma	0 (0%)	3 (3%)	<0.01 (<0.01; 2.40)	0.551
Atrial fibrillation	2 (4.2%)	7 (7%)	0.58 (0.12; 2.80)	0.719
Coronary artery disease	6 (12.5%)	17 (17%)	0.70 (0.26; 1.95)	0.629
Chronic heart failure	1 (2%)	7 (7%)	0.28 (0.02; 1.63)	0.273
COPD	3 (6.25%)	11 (11%)	0.54 (0.16; 1.92)	0.550
ESRD	7 (14%)	9 (9%)	1.65 (0.62; 4.56)	0.404
Hyperlipidemia	13 (27.1%)	36 (36%)	0.66 (0.32; 1.42)	0.352
Hypertension	22 (45.8%)	71 (71%)	0.35 (0.18; 0.71)	0.004[b]
Obesity	27 (56.25%)	40 (40%)	1.93 (0.97; 3.96)	0.078[b]
Smoker	1 (2.1%)	1 (1%)	2.11 (0.11; 40.36)	0.545
Positive chest x-ray	46 (95.8%)	87 (87%)	3.44 (0.82; 15.76)	0.145
Discretionary treatments[a]				
Antibiotics	43 (89.6%)	94 (94%)	0.55 (0.15; 1.71)	0.336
Antivirals	13 (27.1%)	6 (6%)	2.58 (0.88; 7.55)	0.001[b]
Corticosteroids	43 (89.6%)	78 (78%)	2.43 (0.87; 6.20)	0.112
Convalescent plasma	2 (4.2%)	4 (4%)	1.04 (0.19; 4.61)	>0.999

Data are reported as n (%), mean ± standard deviation, or median (interquartile range) [range]. Effect sizes are reported as mean differences (95% CI), Hodges-Lehmann differences (95% CI), or odds ratios (95% CI). P-values were computed using unpaired t-tests (normally distributed data), Mann-Whitney *U* test (nonparametric data), or Fisher's exact test (dichotomous data).
COPD = Chronic obstructive pulmonary disease; ESRD = End-stage renal disease; HCT = Hematocrit; HGB = Hemoglobin; WBC = White blood cell count.
[a] Some patients did not receive treatments on day 1. Within the NAG group, one patient received antibiotics on day 5, two patients received antivirals on day 2 and one patient received antivirals on day 3, and three patients received corticosteroids on day 2. All patients in the control group received medications starting on day 1.
[b] Statistically significant.

[c] Disease severity was assessed based on the World Health Organization [WHO] Ordinal Scale for Clinical Improvement [28]; see Table S1.

Table 2
Univariate comparisons of primary and secondary outcomes between NAG treatment and control groups.

Outcome	NAG (n = 48)	Control (n = 100)	Comparison	p-value
Primary				
Intubation	8 (16.7%)	25 (25%)	0.60 (0.26; 1.48)	0.297
Hospital LOS	7 (5–11.75) [2–31]	7.5 (4–17) [0–59]	0.0 (−1.0; 3.0)	0.643
Mortality	6 (12.5%)	28 (28%)	0.37 (0.15; 0.91)	0.039[b]
Secondary				
ICU admission	11 (22.9%)	36 (36%)	0.53 (0.25; 1.19)	0.133
ICU LOS[c]	2.5 (0.75–14.75)	9.0 (2.25–21.5)	4.0 (−1.0; 12.0)	0.092
Duration of oxygen use (days)[a]	7.0 (5–12) [2–31]	7.0 (3–15) [1–53]	0.0 (−2.0; 2.0)	0.834
Hospice initiation[d]	0 (0%)	4 (5.6%)	<0.00 (<0.00; 1.52)	0.149
Poor clinical outcome	6 (12.5%)	32 (32%)	0.30 (0.12; 0.80)	0.015[b]

Data are reported as n (%) or median (IQR) [range]. Effect sizes are reported as Hodges-Lehmann differences (95% CI) or odds ratios (95% CI). P-values were computed using the Mann-Whitney *U* test or Fisher's exact test.
ICU = Intensive care unit; LOS = Length-of-stay.
[a] The comparison of duration of oxygen use only includes recorded observations from patients that required oxygen support; NAG: n = 46, Control: n = 86.
[b] Statistically significant.
[c] The comparison of ICU length-of-stay only includes recorded observations from patients admitted to the ICU; NAG: n = 10, Control: n = 36.
[d] The hospice initiation population excludes patients that died; NAG: n = 42, Control: n = 72.

Table 3
Multivariate logistic regression, regressing background characteristics and comorbidities against intubation rates.

Variable	OR	95% CI	p-value
(intercept)	<0.01	<0.01; 0.001	<0.001
NAG (ref = control)	0.68	0.19; 2.30	0.541
Time from symptom onset	1.09	1.04; 1.15	<0.001
WBC	1.06	0.96; 1.17	0.257
HLD	3.20	1.09; 10.05	0.038
ESRD	7.48	1.72; 35.31	0.008
Severity	6.59	2.55; 20.83	0.001
SUMMARY	**ROC**	**Adjusted pseudo-R^2**	**p-value**
	0.903	0.529	<0.001

CI = Confidence Interval; ESRD = End-stage renal disease; HLD = Hyperlipidemia; OR = Odds Ratio; ROC = Area under the receiver operating characteristic curve; WBC = White blood cell count.

regression in this study; however, its borderline p-value and significance on univariate analysis are suggestive that larger clinical studies may demonstrate a larger effect. NAG was not a significant independent predictor of intubation on multiple logistic regression. Based on the results from this study, NAG will subsequently move into a randomized controlled trial to continue studying its effectiveness as a therapeutic agent against COVID-19.

Molecular research confirms NAG's involvement with acute and chronic inflammation processes and supports multiple potential mechanisms of action in COVID-19 patients. For example, a study by Petrović et al. investigating variation in immunoglobulin G glycome composition based on the severity of COVID-19 found a significant decrease of bisecting NAG in patients with severe COVID-19 [35]; these results

Table 4
Multivariate linear regression, regressing background characteristics and comorbidities against hospital length-of-stay.

Variable	β-coefficient	95% CI	p-value
(intercept)	0.27	−4.85; 5.39	0.918
NAG (ref = control)	−4.27	−5.67; −2.87	<0.001
Time from symptom onset	0.92	0.85; 0.98	<0.001
HCT	−0.08	−0.17; 0.02	0.108
COPD	−1.44	−3.47; 0.58	0.161
HLD	−0.86	−2.18; 0.46	0.198
Obesity	0.87	−0.47; 2.21	0.203
ESRD	2.54	0.51; 4.57	0.015
Severity	0.70	−0.24; 1.64	0.145
Antibiotics	2.44	−0.10; 4.98	0.059
Antivirals	2.34	0.47; 4.21	0.015
Steroids	−1.91	−3.68; −0.14	0.034
SUMMARY	**RMSE**	**Adjusted R^2**	**p-value**
	0.903	0.870	<0.001

CI = Confidence Interval; COPD = Chronic obstructive pulmonary disease; ESRD = End-stage renal disease; HCT = Hematocrit; HLD = Hyperlipidemia; RMSE = Root mean square error; WBC = White blood cell count.

Table 5
Multivariate logistic regression, regressing background characteristics and comorbidities against mortality.

Variable	OR	95% CI	p-value
(intercept)	<0.01	<0.01; 0.003	<0.001
NAG (ref = control)	0.34	0.09; 1.07	0.081
Age	1.05	1.01; 1.09	0.035
White (ref = Hispanic)	0.42	0.08; 1.76	0.264
Time from symptom onset	1.03	0.99; 1.08	0.146
CAD	2.13	0.61; 7.54	0.235
CHF	4.54	0.59; 33.39	0.132
COPD	2.89	0.56; 14.22	0.188
ESRD	7.11	1.77; 30.64	0.006
Severity	4.51	1.91; 12.33	0.001
Antibiotics	0.18	0.02; 2.59	0.174
Steroids	2.74	0.61; 18.72	0.231
SUMMARY	**ROC**	**Adjusted pseudo-R^2**	**p-value**
	0.875	0.444	<0.001

CAD = Coronary artery disease; CHF = Congestive heart failure; CI = Confidence interval; COPD = Chronic obstructive pulmonary disease; ESRD = End-stage renal disease; OR = Odds Ratio; ROC = Area under the receiver operating characteristic curve.

potentially indicate NAG depletion following increased immunometabolism to fight infection or inflammation. Another study by Krick et al. demonstrated that increased levels of O-linked β-NAG protein modification correlated with increased immune cell secretion in a pathway mediating chronic inflammatory airway diseases [20]. This again suggests that NAG is a crucial component of respiratory immune function.

In terms of human clinical data, one meta-analysis of 15 trials by Vlad et al. found a summary effect size of 0.35 (95% CI: 0.14; 0.56) in favor of glucosamine's effectiveness for osteoarthritis treatment [36], indicating that NAG mediates chronic inflammatory responses. While most NAG research in humans has focused on chronic inflammation, several animal models have confirmed NAG's involvement in acute inflammatory processes. In their study of O-linked β-NAG and traumatic hemorrhage, Nöt et al. found that increasing levels of O-linked β-NAG were associated with improved survival by attenuating inflammation within a 24-h period [21]; similarly, Hirata et al. found that during periods of maximal inflammation, O-linked β-NAG suppressed the acute inflammation response [22]. Although the precise mechanism of action remains as-of-yet undetermined, this cumulative basic science and clinical evidence supports NAG as an anti-inflammatory and adjunctive treatment for COVID-19.

This is the first trial to be published assessing NAG as a therapy for COVID-19 — pending an ongoing, yet-to-be-published investigation of

NAG at the University of California, Irvine [37] — but there has been previous interest in NAG's therapeutic potential in related disease states, as evidenced by a patent pending for analogs of NAG (including D-galactosamine and ranimustine) for the treatment of "low levels of branched N-glycans in a subject in need thereof" [38]. This study also broadly represents the scientific community's growing interest in nutraceuticals as potential COVID-19 treatments [39–41]. Some researchers have postulated that a similar nutraceutical, N-acetylcysteine (NAC), may have protective effects against COVID-19 complications due to its efficacy for other influenza viruses [42,43]. Additional trials will shed light on the efficacy of NAG and other nutraceuticals for use as first-line or adjuvant therapies for COVID-19.

Meanwhile, there are many therapies that have been explored and used to treat COVID-19. These therapies include corticosteroids such as methylprednisolone and dexamethasone [2]; antiviral agents such as lopinavir/ritonavir [4], remdesivir [5], and oseltamivir [6]; immunomodulatory drugs like chloroquine and hydroxychloroquine [7]; serotherapy with antibodies taken from recovered individuals [8]; anticoagulants such as enoxaparin [9]; and inflammatory inhibitors such as tocilizumab, sarilumab [10], and anakinra [11]; yet none of these treatments have drastically reduced the threat of COVID-19. Even with vaccines now in the nascent stages of distribution, there will be challenges and uncertainties ahead before herd immunity may be achieved [44,45], including low uptake among the general population and healthcare workers alike [46–48]. Therefore, it is of utmost importance to continue searching for therapies that may improve COVID-19 prognosis.

4.1. Limitations

The conclusions that can be drawn from this study are limited by several aspects the study design, including its relatively small number of participants, lack of randomization, use of a retrospective control arm, and the temporality of consecutive patients. These limitations were considered necessary costs in order to rapidly provide information during the COVID-19 pandemic; the authors hope to account for these limitations and provide more robust analysis in a subsequent randomized controlled trial. The treatment and control arm patients were admitted to the study during different stages of the pandemic (July 2020–November 2020 for control, November 2020–December 2020 for treatment) so it is possible this introduced bias into the study; fortunately, the institution where the study was taking place was never inundated with COVID-19 cases, so level of care was not affected and mortality was not due to insufficient beds or hospital personnel in any case. Taking place at a single center also reduces the generalizability of this study.

Patients received concomitant standard-of-care therapies according to the discretion of the treating physician, so it is difficult to determine whether patient improvement was due to NAG administration, discretionary therapies, or other factors. Of note, those who received NAG were younger, less likely to have hypertension, and more likely to be administered antivirals, which likely benefitted these patients and contributed to positive outcomes; however, they also were more likely to be obese and had a significantly longer time from symptom onset to admission, which potentially did not benefit NAG patients. With multiple confounding variables at play, it is unclear how these may have impacted results, so conclusions that may be drawn are limited.

Our comparative analysis of mortality rates was also underpowered, achieving statistical power of only 55% on a post-hoc analysis assuming an exact binomial interval. To achieve at least 80% power, both the NAG group and control group would need sample sizes of at least 114 patients – assuming the same observed proportions of mortality between groups, 1:1 allocation ratio, type I error probability of 0.05, and no sample attrition (Fig. S7). A comparative effectiveness randomized controlled trial with matched study arms, with the specifications described above, is needed to further investigate the effect of NAG in COVID-19 patients.

5. Conclusions

NAG shows promise as a first-line treatment against COVID-19 and, when administered orally in 700 mg doses every 12 h, was associated with reduced hospital LOS, ICU admission rates, and death/hospice rates in adults compared to standard care alone. An additional study with a larger set of patients is warranted to evaluate the effect of NAG in COVID-19 patients.

Ethical approval

The research protocol was reviewed and received approval from the institutional review board at MetroWest Medical Center, Framingham Union Hospital, and Leonard Morse Hospital (IRB #2020-106; approved August 26, 2020).

Author contribution

AH conceptualized the study, coordinated data collection, critically revised the manuscript, and oversaw the project. Superior Medical Experts assisted with drafting, editing, and statistical analysis, which was paid for by AH.

Registration of research studies

1. Name of the registry: ClinicalTrials.gov.
2. Unique Identifying number or registration ID: NCT04706416.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): https://clinicaltrials.gov/ct2/show/NCT04706416.

Guarantor

Ameer Hassan, DO.

Consent

Only patients who provided informed consent were included in the study.

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Ethical statement

The research protocol was reviewed and received approval from relevant ethical committees.

Declaration of competing interest

AH has filed a provisional patent for NAG as a treatment for COVID-19. AH reports consulting for Medtronic, Microvention, Stryker, Penumbra, Cerenovus, Genentech, GE Healthcare, Scientia, Balt, Viz.ai, Insera therapeutics, Proximie, NovaSignal and Vesalio; acting as principal investigator for the COMPLETE study – Penumbra, and LVO SYNCHRONISE – Viz.ai; participating in the steering committee/publication committee member for SELECT, DAWN, SELECT 2, EXPEDITE II, EMBOLISE, and CLEAR trials; and acting as a proctor for Pipeline, FRED, Wingspan, and Onyx.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102574.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

[1] COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at, Johns Hopkins University: Johns Hopkins University & Medicine, 2020. https://coronavirus.jhu.edu/map.html.
[2] H. Ledford, Coronavirus breakthrough: dexamethasone is first drug shown to save lives, Nature 582 (7813) (2020) 469, https://doi.org/10.1038/d41586-020-01824-5.
[3] J. Van Paassen, J.S. Vos, E.M. Hoekstra, K.M.I. Neumann, P.C. Boot, S.M. Arbous, Corticosteroid use in COVID-19 patients: a systematic review and meta-analysis on clinical outcomes, Crit. Care 24 (1) (2020), https://doi.org/10.1186/s13054-020-03400-9.
[4] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, et al., A trial of lopinavir–ritonavir in adults hospitalized with severe covid-19, N. Engl. J. Med. 382 (19) (2020) 1787–1799, https://doi.org/10.1056/nejmoa2001282.
[5] C.J. Gordon, E.P. Tchesnokov, J.Y. Feng, D.P. Porter, M. Götte, The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus, J. Biol. Chem. 295 (15) (2020) 4773–4779, https://doi.org/10.1074/jbc.AC120.013056.
[6] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (2020) 507–513, https://doi.org/10.1016/s0140-6736(20)30211-7, 10223.
[7] P. Gautret, J.-C. Lagier, P. Parola, V.T. Hoang, L. Meddeb, M. Mailhe, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents 56 (1) (2020) 105949, https://doi.org/10.1016/j.ijantimicag.2020.105949.
[8] E.M. Bloch, S. Shoham, A. Casadevall, B.S. Sachais, B. Shaz, J.L. Winters, et al., Deployment of convalescent plasma for the prevention and treatment of COVID-19, J. Clin. Invest. 130 (6) (2020) 2757–2765, https://doi.org/10.1172/jci138745.
[9] A. Kollias, K.G. Kyriakoulis, E. Dimakakos, G. Poulakou, G.S. Stergiou, K. Syrigos, Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action, Br. J. Haematol. 189 (5) (2020) 846–847, https://doi.org/10.1111/bjh.16727.
[10] F.M. Buonaguro, I. Puzanov, P.A. Ascierto, Anti-IL6R role in treatment of COVID-19-related ARDS, J. Transl. Med. 18 (1) (2020) 165, https://doi.org/10.1186/s12967-020-02333-9.
[11] G. Cavalli, G. De Luca, C. Campochiaro, E. Della-Torre, M. Ripa, D. Canetti, et al., Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study, Lancet Rheumatol. 2 (6) (2020) e325–e331, https://doi.org/10.1016/s2665-9913(20)30127-2.
[12] COVID-19 Treatment Guidelines, National Institutes of Health, 2021. https://www.covid19treatmentguidelines.nih.gov/.
[13] Coronavirus (COVID-19) Update: FDA Authorizes Drug for Treatment of COVID-19 [press release]. U.S. Food and Drug Administration2021.
[14] W.J. Wiersinga, A. Rhodes, A.C. Cheng, S.J. Peacock, H.C. Prescott, Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19), J. Am. Med. Assoc. 324 (8) (2020) 782, https://doi.org/10.1001/jama.2020.12839.
[15] M.F. McCarty, J.H. O'Keefe, J.J. DiNicolantonio, Glucosamine for the treatment of osteoarthritis: the time has come for higher-dose trials, J. Diet. Suppl. 16 (2) (2019) 179–192, https://doi.org/10.1080/19390211.2018.1448920.
[16] J.-K. Chen, C.-R. Shen, C.-L.N. Liu, Acetylglucosamine: production and applications, Mar. Drugs 8 (9) (2010) 2493–2516, https://doi.org/10.3390/md8092493.
[17] C. Scholtissek, R. Rott, H.D. Klenk, Two different mechanisms of the inhibition of the multiplication of enveloped viruses by glucosamine, Virology 63 (1) (1975) 191–200, https://doi.org/10.1016/0042-6822(75)90384-0.
[18] R.A. Delgadillo, D.A. Vanden Berghe, Inhibition of the multiplication of enveloped and non-enveloped viruses by glucosamine, J. Pharm. Pharmacol. 40 (7) (1988) 488–493, https://doi.org/10.1111/j.2042-7158.1988.tb05283.x.
[19] T. Asselah, D. Durantel, E. Pasmant, G. Lau, R.F. Schinazi, COVID-19: discovery, diagnostics and drug development, J. Hepatol. 74 (1) (2021) 168–184, https://doi.org/10.1016/j.jhep.2020.09.031.
[20] S. Krick, E.S. Helton, S.B. Hutcheson, S. Blumhof, J.M. Garth, R.S. Denson, et al., FGF23 induction of O-linked N-acetylglucosamine regulates IL-6 secretion in human bronchial epithelial cells, Front. Endocrinol. 9 (2018) 708, https://doi.org/10.3389/fendo.2018.00708.
[21] L.G. Nöt, C.A. Brocks, L. Vámhidy, R.B. Marchase, J.C. Chatham, Increased O-linked β-N-acetylglucosamine levels on proteins improves survival, reduces inflammation and organ damage 24 hours after trauma-hemorrhage in rats, Crit. Care Med. 38 (2) (2010) 562–571, https://doi.org/10.1097/ccm.0b013e3181cb10b3.
[22] Y. Hirata, T. Nakagawa, K. Moriwaki, E. Koubayashi, K. Kakimoto, T. Takeuchi, et al., Augmented *O*-GlcNAcylation alleviates inflammation-mediated colon carcinogenesis via suppression of acute inflammation, J. Clin. Biochem. Nutr. 62 (3) (2018) 221–229, https://doi.org/10.3164/jcbn.17-106.
[23] Y. Naraoka, H. Harada, M. Katagiri, H. Yamamura, T. Shirasawa, N-acetyl glucosamine and proteoglycan containing supplement improves the locomotor functions of subjects with knee pain, Drug Disc. Ther. 11 (3) (2017) 140–145, https://doi.org/10.5582/ddt.2017.01019.
[24] S. Salvatore, R. Heuschkel, S. Tomlin, S.E. Davies, S. Edwards, J.A. Walker-Smith, et al., A pilot study of N-acetyl glucosamine, a nutritional substrate for glycosaminoglycan synthesis, in paediatric chronic inflammatory bowel disease, Aliment. Pharmacol. Ther. 14 (12) (2000) 1567–1579, https://doi.org/10.1046/j.1365-2036.2000.00883.x.
[25] Y. Liu, Z. Li, G. Liu, J. Jia, S. Li, C. Yu, Liquid chromatography-tandem mass spectrometry method for determination of N-acetylglucosamine concentration in human plasma, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 862 (1–2) (2008) 150–154, https://doi.org/10.1016/j.jchromb.2007.11.043.
[26] J.N. Hathcock, A. Shao, Risk assessment for glucosamine and chondroitin sulfate, Regul. Toxicol. Pharmacol. 47 (1) (2007) 78–83, https://doi.org/10.1016/j.yrtph.2006.07.004.
[27] R. Agha, A. Abdall-Razak, E. Crossley, N. Dowlut, C. Iosifidis, G. Mathew, STROCSS 2019 Guideline: strengthening the reporting of cohort studies in surgery, Int. J. Surg. 72 (2019) 156–165, https://doi.org/10.1016/j.ijsu.2019.11.002.
[28] Clinical management of COVID-19: living guidance, In: Organization WH, editor, https://www.who.int/publications/i/item/clinical-management-of-covid-192021.
[29] T.W. Anderson, D.A. Darling, A test of goodness of fit, J. Am. Stat. Assoc. 49 (268) (1954) 765–769, https://doi.org/10.1080/01621459.1954.10501232.
[30] J.A. Koziol, R.B. D'Agostino, M.A. Stephens, Goodness-of-Fit techniques, J. Educ. Stat. 12 (412) (1987), https://doi.org/10.2307/1165059.
[31] S.S. Shapiro, M.B. Wilk, An analysis of variance test for normality (complete samples), Biometrika 52 (3–4) (1965) 591–611, https://doi.org/10.1093/biomet/52.3-4.591.
[32] Kolmogorov–Smirnov Test. Springer New York. p. 283-287.
[33] R. Fisher, On the interpretation of $\chi 2$ from contingency tables, and the calculation of P, J. Roy. Stat. Soc. 85 (1) (1922) 87–94, https://doi.org/10.2307/2340521.
[34] J. Baptista, M. Pike, Algorithm AS 115: exact two-sided confidence limits for the odds ratio in a 2×2 table, J. Roy. Stat. Soc. 26 (2) (1977) 214–220, https://doi.org/10.2307/2347041.
[35] T. Petrović, I. Alves, D. Bugada, J. Pascual, F. Vučković, A. Skelin, et al., Composition of the immunoglobulin G glycome associates with the severity of COVID-19, Glycobiology (2020), https://doi.org/10.1093/glycob/cwaa102.
[36] S.C. Vlad, M.P. LaValley, T.E. McAlindon, D.T. Felson, Glucosamine for pain in osteoarthritis: why do trial results differ? Arthritis Rheum. 56 (7) (2007) 2267–2277, https://doi.org/10.1002/art.22728.
[37] M. Demetriou, Reversing COVID-19 Associated ARDS and Cytokine Storm with N-acetylglucosamine. UCI Applied Innovation: Research Translation Group, University of California, Irvine, 2020.
[38] M. Demetriou, J. Dennis, A. Oganesyan, inventorsAnalogs of N-Acetlyglucosamine and Uses Thereof, 2017.
[39] M. Desjarlais, M. Wirth, I. Lahaie, P. Ruknudin, P. Hardy, A. Rivard, et al., Nutraceutical targeting of inflammation-modulating microRNAs in severe forms of COVID-19: a novel approach to prevent the cytokine storm, Front. Pharmacol. 11 (2020), https://doi.org/10.3389/fphar.2020.602999.
[40] M.F. McCarty, J.J. Dinicolantonio, Nutraceuticals have potential for boosting the type 1 interferon response to RNA viruses including influenza and coronavirus, Prog. Cardiovasc. Dis. 63 (3) (2020) 383–385, https://doi.org/10.1016/j.pcad.2020.02.007.
[41] A. Cheudjeu, Correlation of D-xylose with severity and morbidity-related factors of COVID-19 and possible therapeutic use of D-xylose and antibiotics for COVID-19, Life Sci. 260 (2020) 118335, https://doi.org/10.1016/j.lfs.2020.118335.
[42] Z. Shi, C.A. Puyo, N-acetylcysteine to combat COVID-19: an evidence review, Therapeut. Clin. Risk Manag. 16 (2020) 1047–1055, https://doi.org/10.2147/tcrm.s273700.
[43] R.M. Jorge-Aarón, M.P. Rosa-Ester, N-acetylcysteine as a potential treatment for COVID-19, Future Microbiol. 15 (2020) 959–962, https://doi.org/10.2217/fmb-2020-0074.
[44] R.M. Anderson, C. Vegvari, J. Truscott, B.S. Collyer, Challenges in creating herd immunity to SARS-CoV-2 infection by mass vaccination, Lancet 396 (2020) 1614–1616, https://doi.org/10.1016/s0140-6736(20)32318-7, 10263.
[45] K.V. Iserson, SARS-CoV-2 (COVID-19) vaccine development and production: an ethical way forward, Camb. Q. Healthc. Ethics 30 (1) (2021) 59–68, https://doi.org/10.1017/s096318012000047x.
[46] K.O. Kwok, K.K. Li, W.I. Wei, A. Tang, S.Y.S. Wong, S.S. Lee, Influenza vaccine uptake, COVID-19 vaccination intention and vaccine hesitancy among nurses: a

survey, Int. J. Nurs. Stud. 114 (2020) 103854, https://doi.org/10.1016/j.ijnurstu.2020.103854.

[47] V. Grech, C. Gauci, Vaccine hesitancy in the university of Malta faculties of Health sciences, dentistry and medicine vis-à-vis influenza and novel COVID-19 vaccination, Early Hum. Dev. (2020) 105258, https://doi.org/10.1016/j.earlhumdev.2020.105258.

[48] R.D. Goldman, T.D. Yan, M. Seiler, C. Parra Cotanda, J.C. Brown, E.J. Klein, et al., Caregiver willingness to vaccinate their children against COVID-19: cross sectional survey, Vaccine 38 (48) (2020) 7668–7673, https://doi.org/10.1016/j.vaccine.2020.09.084.