AN OBSERVATIONAL COHORT STUDY TO ASSESS N-ACETYLGLUCOSAMINE FOR COVID-19 TREATMENT IN THE INPATIENT SETTING

Protocol Overview and Statistical Analysis Plan

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1.0 PROTOCOL OVERVIEW

1.1 Study Design

This work will be reported in line with STROCSS criteria [1]. This is a single-center, observational study to be 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 has been 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 will be approximately November 2020 to January 2021, aiming to enroll 50 patients to receive NAG.

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

1.2 Inclusion & Exclusion Criteria

Inclusion criteria will remain unchanged for the duration of the study and stipulate that all patients must be ≥18 years old; present with shortness of breath (since local institutional policy only admits patients with shortness of breath); clinical diagnosis of COVID-19 by RT-PCR; hospital admittance due to COVID-19; administered NAG orally as first-line treatment; and no intubation prior to hospitalization and enrollment in the current study. Patients will be excluded if they do not meet criteria above, have an allergy to NAG or shellfish, are currently taking warfarin, or are currently pregnant or lactating.

1.3 Data Collection & Outcomes

Upon admission, the research team will record patient demographics, comorbidities, symptoms, disease severity (as assessed by the World Health Organization [WHO] Ordinal Scale for Clinical Improvement [2]), need for supplemental oxygen, and time from symptom onset until hospital arrival. The research team will also collect 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 will be recorded daily until study exit.

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

Beginning on the study start date, the previous 100 COVID-19-positive consecutive patients admitted to Valley Baptist Medical Center will be retrospectively identified via chart review to serve as the control/comparison arm. Data for these patients will be collected before commencement of the NAG trial. Univariate analysis will be performed for all primary and secondary outcomes, followed by multivariate analysis for primary outcomes and select secondary outcomes that approach significance and have sufficient frequency of occurrence to be meaningful; the main conclusions of this study will be 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 will not be performed for this study. Instead, samples are 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 does not require patient consent for de-identified data and there is sufficient data availability, a larger study arm will be 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.

2.0 STATISTICAL ANALYSIS PLAN

Continuous parameters will be assessed for normality based on cross-validation using Anderson-Darling [3], D'Agostino-Pearson omnibus [4], Shapiro-Wilk [5], and Kolmogorov-Smirnov tests [6]. Comparisons of normally distributed data between groups will be analyzed using unpaired Student's t-tests. Effect sizes from t-tests will be reported as mean differences with 95% confidence intervals (CIs) based on normal approximation. Comparisons of nonparametric data between groups will be analyzed using the Mann-Whitney U test. Effect sizes from nonparametric tests will be reported as Hodge's-Lehman differences (H-L Diff) and their respective 95% CIs. Comparisons of dichotomous data between groups will be analyzed using Fisher's exact binomial test [7]. Effect sizes from Fisher's exact test will be reported as odds ratios (ORs) with 95% CIs computed using the Baptista-Pike method [8].

A correlation matrix showing the strength and direction of correlation between covariates and outcomes will be generated using Spearman's rank correlation test via the 'corrplot' package in R. Spearman's rank correlation will be used to provide a more robust measurement of correlation in the face of high leverage outliers. Simple linear or logistic regressions will be used to evaluate potential predictors of primary and secondary outcomes. Multivariate regression using best subset selection will be 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 will also be evaluated by root mean square error (RMSE) values and multiple logistic regressions will be 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 will also be performed. Best subset selection will be carried out using the 'leaps' package in R. P-values ≤ 0.05 are considered significant for all analyses. All statistics will be performed in RStudio (Version 1.3.959, RStudio, PBC, Boston, MA).

3.0 REFERENCES

[1] Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C, Mathew G. STROCSS 2019 Guideline: Strengthening the reporting of cohort studies in surgery. *Int J Surg* 2019;72:156-65. https://doi.org/10.1016/j.ijsu.2019.11.002

[2] Clinical Management of COVID-19: Living Guidance. In: Organization WH, editor. https://www.who.int/publications/i/item/clinical-management-of-covid-192021.

[3] Anderson TW, Darling DA. A Test of Goodness of Fit. *Journal of the American Statistical Association* 1954;49(268):765-9. https://doi.org/10.1080/01621459.1954.10501232

[4] Koziol JA, D'Agostino RB, Stephens MA. Goodness-of-Fit Techniques. *Journal of Educational Statistics* 1987;12(412). https://doi.org/doi:10.2307/1165059

[5] Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965;52(3-4):591-611. https://doi.org/10.1093/biomet/52.3-4.591

[6] Kolmogorov–Smirnov Test. Springer New York. p. 283-7.

[7] Fisher R. On the Interpretation of $\chi 2$ from Contingency Tables, and the Calculation of P. *Journal of the Royal Statistical Society* 1922;85(1):87-94. https://doi.org/10.2307/2340521

[8] Baptista J, Pike M. Algorithm AS 115: Exact Two-Sided Confidence Limits for the Odds Ratio in a 2 × 2 Table. *Journal of the Royal Statistical Society* 1977;26(2):214-20. https://doi.org/10.2307/2347041