Prognostic Value of Elevated Levels of Plasma N-Acetylneuraminic Acid in Patients With Heart Failure

Chenze Li, MD, PhD*; Mingming Zhao, PhD*; Lei Xiao, MD; Haoran Wei, MD; Zheng Wen, MD, PhD; Dong Hu, MD; Bo Yu, MD; Yang Sun, MD; Jianing Gao, PhD; Xiaqing Shen, BN; Qi Zhang, PhD; Huanhuan Cao, PhD; Jin Huang, MD, PhD; Wei Huang, MD, PhD; Ke Li, MD; Man Huang, MD; Li Ni, MD, PhD; Ting Yu, BN; Liang Ji, MD; Yangkai Xu, PhD; Gang Liu, PhD; Matthew C. Konerman, MD; Lemin Zheng, PhD; Dao Wen Wang, MD, PhD

BACKGROUND: Cardiac sialylation is involved in a variety of physiological processes in the heart. Altered sialylation has been implicated in heart failure (HF) mice. However, its role in patients with HF is unclear, and the potential effect of modulation of cardiac sialylation is worth exploring.

METHODS: We first assessed the association between plasma N-acetylglycerol neuraminic acid levels and the incidence of adverse cardiovascular events in patients with HF over a median follow-up period of 2 years. Next, immunoblot analysis and lectin histochemistry were performed in cardiac tissue to determine the expression levels of neuraminidases and the extent of cardiac desialylation. Finally, the therapeutic impact of a neuraminidase inhibitor was evaluated in animal models of HF.

RESULTS: Among 1699 patients with HF, 464 (27%) died of cardiovascular-related deaths or underwent heart transplantation. We found that the elevated plasma N-acetylglycerol neuraminic acid level was independently associated with a higher risk of incident cardiovascular death and heart transplantation (third tertile adjusted hazard ratio, 2.11 [95% CI, 1.67–2.66], P<0.001). In addition, in cardiac tissues from patients with HF, neuraminidase expression was upregulated, accompanied by desialylation. Treatment with oseltamivir, a neuraminidase inhibitor, in HF mice infused with isoproterenol and angiotensin II significantly inhibited desialylation and ameliorated cardiac dysfunction.

CONCLUSIONS: This study uncovered a significant association between elevated plasma N-acetylglycerol neuraminic acid level and an increased risk of a poor clinical outcome in patients with HF. Our data support the notion that desialylation represents an important contributor to the progression of HF; and neuraminidase inhibition may be a potential therapeutic strategy for HF.

Key Words: heart failure ■ N-acetylglycerol neuraminic acid ■ neuraminidase ■ prognosis ■ risk

Chronic heart failure (HF) is a complex disorder involving various pathophysiological mechanisms, including myocardial apoptosis, inflammation, and altered electrical signaling, which contribute to HF progression characterized by frequent hospitalizations and death. It has been increasingly recognized that aberrant cardiac sialylation represents an important contributor to HF.

Sialylation is a common form of posttranslational modification that involves the addition of a sialic acid molecule to the end of an N- or O-linked glycan on cell surfaces or secreted proteins. Sialic acids, a family of nine-carbon monosaccharides, are uniquely characterized...
WHAT IS NEW?
• The elevated plasma N-acetylneuraminic acid level was associated with a higher risk of incident cardiovascular death and heart transplantation independent of traditional risk factors in patients with heart failure (HF).
• In cardiac tissues from patients with HF, neuraminidase expression was upregulated, accompanied by desialylation.
• Treatment with oseltamivir, a neuraminidase inhibitor, in HF mice significantly inhibited desialylation and ameliorated cardiac dysfunction.

WHAT ARE THE CLINICAL IMPLICATIONS?
• This study uncovered a significant association between elevated plasma N-acetylneuraminic acid level and an increased risk of a poor clinical outcome in patients with HF, suggesting that plasma N-acetylneuraminic acid level may serve as a biomarker for poor progression in patients with HF.
• Our data support the notion that desialylation represents an important contributor to the progression of HF.
• This study described the efficacy of neuraminidase inhibition in the treatment of experimental models of HF, suggesting a feasibility for a novel therapeutic strategy in HF.

Nonstandard Abbreviations and Acronyms

| Abbreviation | Description |
|--------------|-------------|
| HF | heart failure |
| HRs | hazard ratios |
| hs-CRP | high-sensitivity C-reactive protein |
| IQR | interquartile range |
| LDL | low-density lipoprotein |
| Neu | neuraminidase |
| Neu5Ac | N-acetylneuraminic acid |
| NT-proBNP | N-terminal pro-B-type natriuretic peptide |

by a negative charge and hydrophilicity. They can stabilize the conformation of cell surface glycoproteins, such as receptors, and increase cell rigidity. They also serve as binding sites for a variety of pathogens and endogenous ligands, which may be critically involved in signaling activation. In mammals, the most abundant sialic acid species is N-acetylneuraminic acid (Neu5Ac), which accounts for 80% of all sialic acids. In cardiac tissue, cardiomyocytes and endothelial cells are highly sialylated. The presence of a terminal sialic acid in glycoconjugates is vital to the normal cellular function and cardiovascular physiology. The homeostasis of sialylation is determined by sialic acid biosynthesis and conjugation, as well as desialylation. Sialidases, also called Neu (neuraminidases), catalyze the removal of α-glycosidic bond-linked sialic acid residues from glycoproteins and glycolipids. In response to pathological stress, desialylation can lead to disturbance of cellular electrical signaling, platelet aggregation, inflammation, and cardiomyocyte apoptosis.

In patients with HF with a reduced ejection fraction, the relationships between desialylation and HF progression have not been evaluated. In addition, the potential effect of cardiac sialylation modulation through the application of a Neu inhibitor on HF is unknown and needs to be explored. Here, we sought to examine the hypothesis that the levels of free plasma Neu5Ac, produced by Neu through the removal of α-glycosidic bond-linked sialic acid residues from glycoconjugates, may be associated with the progression of HF. Such a finding would suggest interference with the enzyme-producing plasma-free Neu5Ac as a potential therapeutic strategy in HF.

METHODS
The original datasets that support the findings of the present study are available to other researchers upon reasonable request to the corresponding author.

Study Design and Population
The study was approved by the ethics committee of the Tongji Medical College of Huazhong University of Science and Technology and conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Written informed consents were obtained from all individuals. The study cohort was from a previously described multicentre study, which consecutively include patients between January 2008 and June 2018. Enrolled chronic patients with HF with an ejection fraction <40% and New York Heart Association class II, III, or IV symptoms were included in the present study. The cause of HF included in this study comprises ischemia, dilated cardiomyopathy, hypertension, arrhythmia, peripartum cardiomyopathy, hypertrophic cardiomyopathy, and noncompaction of ventricular myocardium. Individuals were excluded if they had cancer, acute myocardial infarction, or unstable angina within 1 month of study enrollment; significant valvar heart disease; or were not optimally managed. Detailed information on age, sex, physical examination, disease history, laboratory testing, and medications were collected upon enrolment with standardized case report forms from the electronic medical chart, and double-entered into Epidata Entry version 3.1 by research investigators. Follow-up was performed by dedicated cardiology nurses through patient interviews, serial telephone contacts, and medical record reviews at 3 months, 6 months, and annually after hospital discharge. For cross-sectional comparison, individuals without significant cardiac disease who were consecutively screened with coronary angiogram and echocardiography at the cardiology department of Tongji hospital from October 2013 to June 2018 served as the study control. This control group was from a case-control genetic study aiming to identify new risk variants for cardiomyopathy.
Full details of the inclusion and exclusion criteria are provided in Table I and II in the Data Supplement. Furthermore, cardiac tissues from patients with HF (n=4) who underwent heart transplantation and from sex- and age-matched individuals (n=5) who died in traffic accidents and without confirmed heart disease were used to evaluate the expressions of Neu and cardiac desialylation.

**Neu5Ac Measurement**
Baseline fasting peripheral blood samples were collected with EDTA-coated anticoagulated tubes, immediately centrifuged at 3000 rpm for 8 minutes to obtain plasma, and frozen at −80°C until analysis. The plasma levels of Neu5Ac were measured as follows: 20 μL of plasma was aliquoted to a 1.5-mL tube and mixed with 80 μL of a 10-μM internal standard comprised of 13C3-Neu5Ac (Cat: NEU-004, Omicron Biochemicals, South Bend) in methanol by vortexing for 1 minute at 4 to 8°C. The supernatant was recovered following centrifugation at 20,000g at 4°C for 10 minutes. Supernatants (70 μL) were analyzed by injection onto a silica column (2.0×150 mm, Luna 5u Silica 100 A; Catalog No. 00F-4274-B0; Phenomenex, Torrance, CA) at a flow rate of 0.5 mL/min using an LC-20AD Shimadzu pump system, SIL-20AXR autosampler interfaced with an API 5500Q-TRAP mass spectrometer (AB SCIEX, Framingham, MA). A discontinuous gradient was generated to resolve the analytes by mixing solvent A (0.1% formic acid and 10 mmol/L ammonium formate in water) with solvent B (0.1% formic acid in acetonitrile). Nitrogen was used as the nebulizer (gas1), heater (gas2), curtain gas, and the collision activation dissociation gas. Detailed parameters of targeted mass spectrometry and liquid chromatography gradients are listed in Table III in the Data Supplement. Analytes were monitored using electrospray ionization in negative-ion mode with multiple reaction monitoring of precursor and characteristic product-ion transitions of Neu5Ac at m/z 308.1→87 and 308.1→170, and 13C3-Neu5Ac at m/z 311.1→87 and 311.1→173, respectively. A standardized curve was used to determine the precise concentration of Neu5Ac. Standard curves were created by using 20 μL of various concentration standards (0–100 μM) and were deemed acceptable if the coefficient of determination (R²) was 0.999. The accuracy of Neu5Ac concentrations was calculated (Table IV in the Data Supplement). Method reproducibility was checked by intraday and interday accuracy using samples at different concentrations (0.5 μM, 1 μM, and 5 μM). The relative SD of repetitive quantification results was calculated (Table V in the Data Supplement). Quality control was also performed with different Neu5Ac concentrations measured every 20 samples. The calculated means and coefficient of variation are listed in Table VI in the Data Supplement.

**Ascertainment of Outcomes**
The primary end point was a composite of cardiovascular death and heart transplantation. The secondary end points included all-cause mortality, the occurrence of worsening HF, and first rehospitalization for cardiovascular causes. Definitions of all end points were ascertained based on the guideline of the American College of Cardiology and the American Heart Association. Cardiovascular death was defined as death attributed to cardiovascular cause, including acute myocardial infarction, sudden cardiac death, HF, stroke, cardiovascular procedure, cardiovascular hemorrhage, and other cardiovascular causes. The occurrence of worsening HF was recognized as presentation for an urgent visit or hospital admission, with a primary diagnosis of HF by cardiologists. Rehospitalization refers to readmission to the hospital for more than a day stay during the follow-up period. Rehospitalization for cardiovascular cause incorporated unstable or stable angina or atypical chest pain, stroke, nonfatal cardiac arrest, arrhythmia, cardiovascular surgery, major hemorrhage, and HF. The adjudication of clinical end points was systematically performed by 2 independent physicians. For all patients, the survival time was defined as from the enrollment to the first occurrence of primary and secondary end points or the last available follow-up.

**Animals**
Male C57BL/6 mice, 8 to 10 weeks of age (weighing 24±1 g), were purchased from Beijing HFK Bioscience Company, Beijing, China. HF with a reduced ejection fraction was induced by isoproterenol (Sigma-Aldrich, St. Louis, MO) at a dosage of 30 mg/kg per day (containing 0.002% ascorbic acid) or angiotensin II (MedChemExpress, Monmouth Junction, NJ) at a dosage of 2.16 mg/kg per day for 28 days using subcutaneously implanted osmotic pumps (Alzet, Model 1004, Cupertino, CA), as previously described. For the vehicle-control infusion group, an equal volume of saline was used for infusion through the subcutaneously implanted osmotic pumps. Oseltamivir phosphate (50 mg/kg per day, MeilunBio, Dalian, Liaoning, China) or saline was administered intraperitoneally to mice beginning 14 days after isoproterenol infusion or angiotensin II and continued until the end of the protocol (Figure I in the Data Supplement). The number of mice in each group ranged from five to eight. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology.

**Echocardiography and Hemodynamic Detection**
Transthoracic echocardiography was performed using a high-resolution imaging system with a 30-MHz high-frequency scanhead (VisualSonics Vevo770, VisualSonics, Toronto, Canada), as described previously. A pressure-volume catheter (Millar 1.4F, SPR 835, Millar Instrument, Inc, Houston, TX) was inserted into the left ventricle through the carotid artery to measure intraventricular pressure and volume, as described previously.

**Statistical Analyses**
The Kolmogorov-Smirnov test was used to determine the distribution of continuous data, reported as mean±SD if normally distributed or median (interquartile range [IQR]). A comparison of means was performed with Student’s t test or a 1-way ANOVA if the distribution was normal; otherwise, Wilcoxon rank-sum test or the Kruskal-Wallis test was used. Categorical data are summarized as numbers (percentages). Percentage distributions between groups were compared using χ² tests. Kaplan-Meier survival plots were used to show the relationship of Neu5Ac with clinical end points according to tertiles, and log-rank tests were used for statistical assessments. Univariate and multivariable
Cox hazards regressions were performed to determine hazard ratios (HRs) and 95% CIs stratified with regard to Neu5Ac as tertiles. The differential associations of Neu5Ac with the risk of the primary end point across all subgroups were tested by use of a test for interaction. Given that multivariable Cox regression require a complete dataset, missing values in the models were imputed by the MICE package (Multiple Imputation by Chained Equations; R version 3.4.1, Vienna, Austria) to decrease I/II false rate. The imputations were carried out under the assumption that the missing data were missing at random. In the MICE procedure, each variable with missing data is imputed according to its distribution. 23 The missing variables and the goodness of fit for missing data imputation are illustrated in Figures II and III in the Data Supplement. Spearman correlations were calculated between Neu5Ac and cardiac and inflammatory markers. Harrell C-index, net reclassification indexes, and integrated discrimination improvements were used to evaluate the incremental predictive ability of Neu5Ac for predicting the risk of the primary end point in patients with HF.

All comparisons were 2-sided, and \( P \text{ value} <0.05 \) was considered statistically significant. R (version 3.4.1, Vienna, Austria) was used to perform all analyses. Description of the other methods is available in the Data Supplement.

**RESULTS**

**Participant Characteristics**

In the study cohort, a total of 1699 patients with a diagnosis of HF fulfilling the inclusion and exclusion criteria

| Median (range, μM) | Overall (n=1699) | Tertile 1 (n=566) | Tertile 2 (n=567) | Tertile 3 (n=566) | \( P \) value |
|-------------------|------------------|------------------|------------------|------------------|-------------|
| Demographics      |                  |                  |                  |                  |             |
| Age, y            | 59 [48–68]       | 58 [47–66]       | 58 [47–67]       | 62 [51–69]       | <0.001      |
| Male, n (%)       | 1206 (71)        | 399 (71)         | 400 (71)         | 407 (72)         | 0.8         |
| History of diabetes mellitus, n (%) | 424 (25) | 136 (24) | 117 (21) | 171 (30) | 0.001 |
| History of hypertension, n (%) | 1024 (60) | 367 (65) | 307 (54) | 350 (62) | 0.001 |
| History of stroke, n (%) | 87 (5) | 26 (5) | 26 (5) | 35 (6) | 0.4 |
| Ischemic cause, n (%) | 527 (31) | 172 (30) | 153 (27) | 202 (36) | 0.006 |
| Smoking, n (%)    | 691 (41)         | 222 (39)         | 233 (41)         | 236 (42)         | 0.7         |
| Drinking, n (%)   | 395 (23)         | 145 (26)         | 125 (22)         | 125 (22)         | 0.3         |
| NYHA class at enrollment, n (%) |                  |                  |                  |                  | 0.001       |
| II                | 512 (30)         | 198 (35)         | 165 (29)         | 149 (26)         |             |
| III               | 724 (43)         | 238 (42)         | 256 (45)         | 230 (41)         |             |
| IV                | 463 (27)         | 130 (23)         | 146 (26)         | 187 (33)         |             |

**Clinical testing**

| Systolic pressure, mm Hg | 121 [109–138] | 122 [110–137] | 120 [107–136] | 123 [108–140] | 0.3 |
| Diastolic pressure, mm Hg | 78 [69–89] | 78 [69–88] | 78 [70–89] | 79 [68–90] | 1 |
| Heart rate, beats/min | 86 [74–100] | 85 [72–100] | 86 [74–100] | 87 [76–102] | 0.3 |
| LVEDD, mm | 64 [58–69] | 65 [59–70] | 64 [59–70] | 63 [57–69] | 0.02 |
| Ejection fraction, % | 30 [24–35] | 31 [25–36] | 29 [23–35] | 30 [23–35] | 0.001 |
| NT-proBNP, pg/mL | 3806 [1670–8665] | 2640 [1158–5267] | 3533 [1685–7862] | 6423 [2485–15212] | <0.001 |
| hs-CRP, mg/L | 5.2 [1.8–16.4] | 3.2 [1.1–10.4] | 4.4 [1.9–11.1] | 9.9 [3.8–29.5] | <0.001 |
| Creatinine, μmol/L | 91 [74–118] | 77 [65–92] | 87 [73–103] | 116 [91–165] | <0.001 |
| ALT, U/L | 24 [16–43] | 24 [16–43] | 24 [16–44] | 24 [14–42] | 0.4 |
| AST, U/L | 26 [20–40] | 25 [19–39] | 27 [20–39] | 27 [19–42] | 0.4 |
| HDL cholesterol, mmol/L | 0.9 [0.7–1.1] | 1.0 [0.8–1.2] | 0.9 [0.7–1.1] | 0.9 [0.7–1.1] | <0.001 |
| LDL cholesterol, mmol/L | 2.4 [1.9–2.9] | 2.4 [1.9–2.9] | 2.4 [1.9–2.8] | 2.3 [1.7–3.0] | 0.6 |

**Baseline medication**

| Diuretics, n (%) | 1454 (86) | 480 (85) | 484 (85) | 490 (87) | 0.7 |
| ACE inhibitor/ARB, n (%) | 1420 (84) | 478 (85) | 505 (89) | 437 (77) | <0.001 |
| Beta-blocker, n (%) | 1116 (66) | 387 (68) | 393 (6) | 336 (59) | 0.001 |
| Spironolactone, n (%) | 1348 (79) | 456 (81) | 454 (80) | 438 (77) | 0.4 |

Continuous data are displayed as median (25% and 75% quartiles). The \( P \) values were calculated with \( \chi^2 \) test for the categorical data and Kruskal-Wallis test for continuous data. ACE indicates angiotensin-converting enzyme; ALT, alanine transaminase; ARB, angiotensin receptor blockers; AST, aspartate aminotransferase; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LVEDD, left ventricular end-diastolic dimension; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

*Circ Heart Fail.*, 2021;14:e008459. DOI: 10.1161/CIRCHEARTFAILURE.121.008459 November 2021 1214
were included. At baseline, 86% of patients were treated with diuretics, 84% with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, 79% with mineralocorticoid-receptor antagonists, and 66% with beta-blockers. We summarized the baseline demographic and clinical characteristics of patients according to Neu5Ac tertiles (Table 1). Compared with lower Neu5Ac groups, patients with higher Neu5Ac levels were older and had a higher prevalence of class IV New York Heart Association functional classification and higher NT-proBNP (N-terminal pro-B-type natriuretic peptide), creatinine, and hs-CRP (high-sensitivity C-reactive protein) levels. The plasma Neu5Ac level was positively correlated with NT-proBNP (Spearman correlation coefficients \( r_s = 0.36, P < 0.001 \)) and hs-CRP levels \( (r_s = 0.30, P < 0.001) \). For cross-sectional comparisons, 1700 were recruited to the control group (median age, 56 years old; 45% male), among which 8%, 31%, 6% had a history of diabetes, hypertension, and stroke, respectively. The demographics are shown in Table VII in the Data Supplement.

**Plasma Neu5Ac Levels and Clinical Outcomes**

The median Neu5Ac level was 0.94 μM (IQR, 0.69–1.40 μM) in patients with HF, which was significantly higher than that in the control group (0.61 μM; IQR, 0.48–0.79 μM; \( P < 0.001 \); Figure 1A). As a continuous variable, elevated Neu5Ac level was associated with a 1.12-fold increased risk of HF after adjustment for age, sex, systolic blood pressure, diabetes, LDL (low-density lipoprotein) cholesterol, high-density lipoprotein cholesterol, and smoking (95% CI, 1.11–1.14, \( P < 0.001 \)). Over a median 24-month follow-up, 483 patients with HF (28%) died; 427 (25%) of whom suffered from cardiovascular-related deaths, and 37 patients (2%) underwent heart transplantation. Therefore, 27% (464/1699) of patients fulfilled the primary end point. Cardiovascular rehospitalization occurred in 594 (35%) patients, and 566 patients (33%) had worsening HF. Kaplan-Meier analyses of Neu5Ac levels represented a graded enhanced risk for the composite of cardiovascular death and heart transplantation (Figure 1B), as well as associations with every secondary end point (Figures IV through VI in the Data Supplement). An increase in Neu5Ac levels was associated with an increased incidence of primary adverse events after adjustment for traditional risk factors (adjusted HR, 1.66 [95% CI, 1.45–1.90] per SD; \( P < 0.001 \)). We then categorized patients with HF into 3 groups according to Neu5Ac tertiles. For the primary end point and every secondary end point, the multivariable-adjusted HR (95% CI) of Neu5Ac tertiles are presented in Table 2. Furthermore, the associations of Neu5Ac levels with the composite rate of cardiovascular death and heart transplantation were assessed in several prespecified subgroups. The effect of elevated Neu5Ac levels on the risk of cardiovascular death and heart transplantation were found to be significantly different between patients

![Figure 1. Associations of plasma N-acetylneuraminic acid (Neu5Ac) levels with heart failure (HF) in the study population.](image-url)

**A.** Comparison of Neu5Ac levels between patients with HF (n=1699) and controls (n=1700). The \( P \) value was calculated using Wilcoxon rank-sum test. **B.** Kaplan-Meier curve of the primary end point incidence stratified by Neu5Ac tertiles in patients with HF. The \( P \) value was calculated using the log-rank test.
without and with a history of hypertension (HR, 1.86 [95% CI, 1.42–2.43] versus HR, 3.47 [95% CI, 2.17–5.54]; \( P \leq 0.019 \) for interaction). It is speculated that in patients with HF and hypertension, elevated plasma Neu5Ac level arose partly from the desialylation of IgG and FcyRIIB in the endothelium\(^2^4\) but was not fully from cardiomyocytes, which reduced the effect of Neu5Ac on their prognosis compared with patients with HF but without hypertension. However, no significant interactions were observed for other prespecified subgroups (Figure 2A). To examine the hypothesis that concurrent assessment of Neu5Ac and NT-proBNP enhanced long-term risk stratification among patients with HF, patients were stratified based on Neu5Ac tertiles and NT-proBNP median, and the findings are illustrated in Figure 2B. In addition, we calculated the discrimination and reclassification measures with the addition of Neu5Ac levels to explore the clinical implications of our findings to predict the composite of the primary end point. The results showed that the addition of Neu5Ac to a model of traditional risk factors and NT-proBNP significantly improved the C-index (0.684 [95% CI, 0.659–0.710] to 0.695 [95% CI, 0.670–0.720]; \( P < 0.001 \)) and net reclassification (net reclassification index, 13.5%; \( P = 0.009 \); integrated discrimination improvements, 0.4%, \( P = 0.04 \)). Furthermore, traditional risk factors combined with hs-CRP yielded a C-index of 0.659 (95% CI, 0.632–0.685), increasing to 0.684 (95% CI, 0.659–0.710) with the addition of Neu5Ac (\( P < 0.001 \)); inclusion of Neu5Ac also increased the net reclassification indices (net reclassification index, 29.4%; \( P < 0.001 \); integrated discrimination improvements: 1.5%; \( P < 0.001 \)).

### Upregulation of Neu Expression and Desialylation During HF

The plasma Neu level was significantly increased in patients with HF (median, 3.8 U/mL; IQR, 2.9–4.7 U/mL) in comparison with the controls (median: 2.0 U/mL; IQR, 1.2–2.7 U/mL; \( P < 0.001 \); Figure 3). Based on subcellular localization and substrate specificities, 4 distinct human Neu have been identified: Neu1, Neu2, Neu3, and Neu4.\(^2^5\) Neu proteins were measured in human cardiac

### Table 2. Long-Term Outcomes According to Tertile of Neu5Ac Level in the Study Cohort for the Comparison With Tertile 1

|                    | Tertile 2 | Tertile 3 | \( P \) value for trend |
|--------------------|-----------|-----------|------------------------|
| **Primary composite outcome** |           |           |                        |
| Unadjusted         | 1.46 (1.14–1.87), 0.003 | 2.37 (1.89–2.98), <0.001 | <0.001 |
| Adjusted model 1*  | 1.48 (1.15–1.89), 0.002 | 2.23 (1.77–2.80), <0.001 | <0.001 |
| Adjusted model 2†  | 1.44 (1.12–1.84), 0.004 | 2.11 (1.67–2.66), <0.001 | <0.001 |
| Adjusted model 3‡  | 1.40 (1.09–1.80), 0.007 | 1.93 (1.52–2.48), <0.001 | <0.001 |
| **Secondary outcomes** |           |           |                        |
| Death from any cause |           |           |                        |
| Unadjusted         | 1.30 (1.03–1.66), 0.03 | 2.24 (1.80–2.79), <0.001 | <0.001 |
| Adjusted model 1*  | 1.32 (1.04–1.68), 0.02 | 2.06 (1.65–2.57), <0.001 | <0.001 |
| Adjusted model 2†  | 1.29 (1.01–1.64), 0.04 | 1.96 (1.57–2.45), <0.001 | <0.001 |
| Adjusted model 3‡  | 1.26 (0.99–1.61), 0.06 | 1.76 (1.39–2.21), <0.001 | <0.001 |
| First hospitalization for cardiovascular causes |           |           |                        |
| Unadjusted         | 1.08 (0.89–1.32), 0.4 | 1.37 (1.12–1.66), 0.002 | 0.002 |
| Adjusted model 1*  | 1.08 (0.89–1.33), 0.4 | 1.30 (1.07–1.58), 0.009 | 0.01 |
| Adjusted model 2†  | 1.08 (0.88–1.32), 0.5 | 1.28 (1.05–1.56), 0.01 | 0.01 |
| Adjusted model 3‡  | 1.06 (0.87–1.30), 0.6 | 1.14 (0.92–1.40), 0.2 | 0.2 |
| Recurrence of heart failure |           |           |                        |
| Unadjusted         | 1.16 (0.94–1.42), 0.2 | 1.41 (1.15–1.72), 0.001 | 0.001 |
| Adjusted model 1*  | 1.16 (0.94–1.42), 0.2 | 1.31 (1.07–1.60), 0.009 | 0.009 |
| Adjusted model 2†  | 1.13 (0.92–1.39), 0.3 | 1.27 (1.04–1.56), 0.02 | 0.02 |
| Adjusted model 3‡  | 1.09 (0.89–1.35), 0.4 | 1.11 (0.89–1.38), 0.3 | 0.3 |

The HRs were reported for tertile 2 or tertile 3 with tertile 1 as reference. HR indicates hazard ratio; and Neu5Ac, N-acetylneuraminic acid.

*Adjusted for age and sex.
†Adjusted for traditional risk factors, including age, sex, systolic blood pressure, history of diabetes, ischemic cause, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and smoking.
‡Adjusted for age, sex, systolic blood pressure, history of diabetes, ischemic cause, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking, NT-proBNP (N-terminal pro-B-type natriuretic peptide), high-sensitivity C-reactive protein, creatinine, the use of diuretics, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, beta-blockers, and spironolactone.
tissue using Western blot analysis. Neu3 expression was highly upregulated and Neu1 expression tended to be increased in patients with HF rather than Neu2 and Neu4 expression compared with controls (Figure 4A). Next, we visualized the α-(2,6)-glycosidic bond-linked sialic acid in human cardiac tissues. A significant reduction of terminal sialic acid linkages (fluorescence signals) was observed in HF samples compared to controls (Figure 4B). These data suggested that the desialylation of cardiac tissues was involved in the elevation of both plasma Neu and Neu5Ac levels.

Oseltamivir Phosphate Improves Impaired Cardiac Function in HF Mice

After 2 weeks of isoproterenol or angiotensin II infusions, mice were randomly assigned to receive oseltamivir phosphate treatment or saline. The results showed that HF mice had a higher plasma Neu5Ac level than controls at four weeks postinfusion. After oseltamivir phosphate treatment, the plasma levels of Neu5Ac decreased in HF mice with oseltamivir phosphate treatment compared to saline treated HF mice (Figure 5A and Figure VIIA). The cardiac function, including ejection fraction, fractional shortening and left ventricular internal diameter at systole were improved by oseltamivir phosphate in isoproterenol-induced HF (Figure 5B through 5E). Invasive pressure-volume analysis further suggested that administration of oseltamivir phosphate resulted in a restoration of cardiac function as evidenced by the markedly enhanced dP/dt max in isoproterenol-induced HF mice with oseltamivir phosphate treatment (Figure 5F). Moreover, the levels of α-(2,6)-glycosidic bond-linked sialic acid were increased and invading macrophages were inhibited in HF cardiac tissues after oseltamivir phosphate treatment (Figure 5G, Figure VIIH). For angiotensin II–induced HF, improvements in cardiac function, including ejection fraction, fractional shortening, stroke volume, cardiac output, left ventricular internal diameter at systole, and dP/dt max

Figure 2. Hazard ratios (HRs) associated with N-acetylneuraminic acid (Neu5Ac) levels in subgroups of patients with heart failure (HF).

A, Comparing tertile 1 and tertile 3 of plasma Neu5Ac levels for the primary end point incidence stratified by baseline characteristics. B, Plot of adjusted hazard ratio for the primary end point incidence stratified by Neu5Ac tertiles (cutoff values 0.77 and 1.19 µM) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) median (3805 pg/mL) in patients with HF. HRs and 95% CI were calculated with multivariable Cox regression adjusted for age and sex. Cr indicates creatinine; hs-CRP, high-sensitivity C-reactive protein; and Ref, reference.
were also observed after oseltamivir phosphate treatment (Figure VIIB through VIIG in the Data Supplement). These findings collectively indicated that oseltamivir phosphate had positive protective effects on HF.

**DISCUSSION**

Our study evaluated a large cohort of patients with HF with reduced ejection fraction and uncovered a significant relationship between elevated plasma Neu5Ac levels and increased long-term cardiovascular adverse events independent of traditional risk factors, as well as correlations between Neu5Ac levels and both cardiac and inflammatory markers. Furthermore, we provided evidence for the therapeutic effect of a Neu inhibitor on isoproterenol and angiotensin II–induced HF in mice through decreasing the plasma Neu5Ac level and inhibiting inflammation (Figure 6). To our best knowledge, this is the first human study to report a relationship between the plasma Neu5Ac level and the status and progression of patients with HF.

The mechanisms contributing to the association of elevated plasma Neu5Ac level with a poor clinical outcome in the setting of HF are likely multifactorial. First, one study by Zhang et al revealed a mechanistic link between elevated Neu5Ac levels and myocardial dysfunction for its direct cytotoxicity to cardiomyocytes by inducing apoptosis. Furthermore, a previous study reported that the level of Neu1 is upregulated in invading immune cells and locally in cardiomyocytes after ischemia-reperfusion, manifested as increased inflammation and enhanced cardiomyocyte hypertrophy, which suggest the pathogenic role of Neu1 in HF. Presumably, the elevated Neu5Ac level, produced by Neu through removing α-glycosidic bond-linked sialic acid residues from glycoproteins and glycolipids, maybe an intermediate outcome of disease progression. Besides, there is increasing evidence to implicate systemic desialylation in coronary atherogenesis. During desialylation, inflammatory cells attach more effectively to the endothelial surface. Simultaneously, reduced sialic acid content on the platelet membrane and LDL lead to platelet aggregation and accumulation of cholesteryl esters and neutral lipids in smooth muscle cells, which can exacerbate atherosclerosis progression. Although we observed that elevated plasma Neu5Ac levels were associated with an increased risk of the primary end point in patients with HF with a history of coronary heart disease, the same association was also observed in nonischemic HF, indicating that other underlying mechanisms of ischemia could be involved. In addition, previous findings have shown that decreased exposure of Neu5Ac’s cellular surface facilitates the recognition of apoptotic cellular debris and engulfment of apoptotic material by the circulating phagocytes. The increased free Neu5Ac level may represent one of the indicators for worsening HF. However, further studies should be conducted to elucidate the mechanistic link between desialylation and HF progression.

![Figure 4. Upregulation of Neu (neuraminidase) levels and desialylation in cardiac tissue of patients with heart failure (HF). A, Western blot analyses for Neu (B) α-(2,6)-linked sialic acid detected by lectin histochemistry in cardiac tissue from controls (n=5) and patients with HF who underwent heart transplantation (n=4). Protein expression and fluorescence intensity are quantified by Image J. Data are presented as mean±SEM and analyzed by Student t test; scale bar=50 µm.](image-url)
Furthermore, we linked the Neu5Ac level with cardiac and inflammatory markers. Whether the changes of plasma Neu5Ac levels in HF indirectly or directly cause cardiac injury and inflammation remain to be demonstrated, but we did note modest yet positive correlations of plasma Neu5Ac levels with NT-pro-BNP levels and hs-CRP levels. Moreover, the prognostic value of the plasma Neu5Ac level in patients with HF is independent of NT-proBNP. Additionally, C statistics further suggested that Neu5Ac provided added prognostic value to traditional cardiovascular risk factors and hs-CRP to project the cardiovascular outcome. Sialic acid has been regarded as a marker of inflammation in various pathological conditions.27,31,32 One study investigated the role of inflammatory markers in chronic diseases, showing that sialic acid had the highest discrimination ratio compared with other acute phase markers, although there was a large within-individual variability in CRP level.23 Regan et al34 also suggested that sialic acid is associated with a more sustained inflammatory response, compared with CRP. Consistent with these studies, our study demonstrated a quantitative impact of Neu5Ac in predicting adverse cardiovascular outcomes at different increments.

The elevated Neu5Ac level and Neu expression in patients with HF in the current study suggests that inhibiting desialylation may be useful in the treatment of HF. Oseltamivir phosphate (Tamiflu), a Neu inhibitor, is stockpiled worldwide for prophylaxis and treatment for the pandemic of a new influenza strain.35 Other studies have suggested that inhibition or modulation of Neu activity may be repurposed for therapy other than influenza.36–38 In the current study, we observed that plasma-free Neu5Ac levels decreased and cardiac tissue α-(2,6)-glycosidic bond-linked sialic acid increased after oseltamivir phosphate treatment in HF mice compared with those treated with saline. Importantly, cardiac

**Figure 5.** Oseltamivir phosphate (OS) inhibits desialylation and causes reversal of cardiac function in isoproterenol (ISO)-induced heart failure (HF) mice.

A. Free N-acetylneuraminic acid (Neu5Ac) concentrations in the plasma detected by liquid chromatograph-mass spectrometry (n=5–8 mice per group). Cardiac function at 4 wk (B–F). B. Representative M-mode echocardiogram. C. Statistics of ejection fraction (EF). D. Statistics of fractional shortening (FS). E. Statistics of left ventricular internal diameter (LVID) by echocardiography (n=5–8 mice per group). F. Quantification of dP/dt\(_{\text{max}}\) measured by aortic catheterization (n=3–6 mice per group). G. Representative histochemistry staining of α-(2,6)-linked sialic acid (SNA [Sambucus nigra lectin]; green) and F4/80 (ADGRE1 [adhesion G protein-coupled receptor E1]; red) in cardiac tissues. Data are presented as mean±SEM and analyzed by 1-way ANOVA with Bonferroni test among 4 groups. Vehicle refers to the mice infused with an equal volume of saline through subcutaneously implanted osmotic pumps. DAPI indicates 4′,6-diamidino-2-phenylindole; dP/dt\(_{\text{max}}\), peak rate of pressure increase; and LVID; s, LVID at systole.
function improved following oseltamivir phosphate treatment, indicated by cardiac parameters, including ejection fraction, fractional shortening, stroke volume, cardiac output, left ventricular internal diameter, and \( \frac{dP}{dt_{\text{max}}} \). Taken together, oseltamivir phosphate may offer a promising therapeutic benefit in HF by inhibiting desialylation.

Our findings have several mechanistic and clinical implications. First, our study supports the hypothesis that aberrant desialylation may contribute to the pathogenies of HF, as evidenced by increased plasma Neu5Ac levels and upregulated Neu expression in patients with HF. Second, this is the first study to demonstrate that elevated Neu5Ac levels were associated with a poor prognosis in patients with HF, independent of traditional risk factors, and biomarkers, including NT-proBNP, creatinine, hs-CRP, and the use of medications, indicating additional underlying mechanisms. However, the specific underlying mechanisms are not yet fully established and warrant further studies. Finally, this study described the effect of oseltamivir phosphate to inhibit desialylation and its efficacy in the treatment of experimental models of HF. Maisel\(^{26}\) suggested that a useful therapeutic target for HF should have underlying pathophysiological relevance, but also be able to be monitored by treatment. Except for natriuretic peptide, the most important diagnostic and prognostic index, a variety of markers have been proposed related to HF. However, the use of these markers to guide therapy is either impractical or ineffective.\(^{40}\) From our preclinical animal model results, it is speculated that plasma Neu5Ac level may serve as a biomarker to monitor HF progression and treatment efficacy, as well as to stratify patients requiring Neu inhibitor therapy. However, its usefulness in clinical practice needs further exploration in patients with HF using random clinical trials in the future.

The present study has several limitations: First, there are 4 types of Neu in mammals: Neu1, Neu2, Neu3, and Neu4,\(^{25}\) yet our study only measured the total Neu concentrations in plasma. However, the expression of the 4 Neu in human cardiac tissues were detected by Western blot analysis. The results showed that the upregulation of Neu1 and Neu3 might be responsible for the total increase in Neu. Another potential limitation is that the specific Neu targeted by oseltamivir phosphate was not confirmed. However, oseltamivir phosphate has previously been reported to have a direct effect on endogenous Neu, including Neu1 and Neu3.\(^{41}\) Finally, both mouse models of isoproterenol and angiotensin II have been widely used to mimic advanced HF during preclinical testing,\(^{20,42}\) although the causes of human HF are far more complex conditions, involving coronary artery disease, hypertension,
disease, and cardiomyopathy. Further exploration of the clinical effectiveness of oseltamivir phosphate in patients with HF warrants large-scale clinical trials.

In conclusion, patients with HF had elevated Neu5Ac expression and plasma Neu5Ac levels in comparison to those without HF. Elevated plasma Neu5Ac levels portended long-term poor prognosis independent of traditional risk factors in patients with HF. Moreover, a Neu inhibitor was effective in treating HF mice, presenting an opportunity for a novel therapeutic strategy in HF.

ARTICLE INFORMATION
Received February 6, 2021; accepted August 25, 2021.

Affiliations
Department of Cardiology, Zhongnan Hospital of Wuhan University, Institute of Myocardial Injury and Repair, Wuhan University, China (C.L.), Division of Cardiology, Department of Internal Medicine and Heart: Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Tongji Hospital (C.L.), Xinhua Hospital Affiliated to Shanghai Jiao Tong University (J.X.); and the Key Laboratory of Cardiomyocyte Molecular Biology and Regulatory Peptides, Beijing Key Laboratory of Cardiovascular Receptors Research, Beijing, China (M.Z.), The Institute of Cardiovascular Sciences, Peking University, Third Hospital, NHIC Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Beijing Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Beijing, China (L.X.), and the Advanced Innovation Center for Human Brain Protection, The Capital Medical University, Beijing (L.Z.).

Acknowledgments
We thank Prof Ybin Wang for English polishing and Prof Frank B. Hu for his suggestions on population-based studies.

Sources of Funding
This research was supported by grants from the National Key R&D Program of China (2017YFC0909400 and 2016YFC0903000), National Natural Science Foundation of China (91432003, 81700413, 91639108, 81770727, 81800356, and 81970425), Shanghai Municipal Science and Technology Major Science and Technology Project (2018SHZDXK01), and National Postdoctoral Program for Innovative Talents (BX202000022).

Disclosures
None.

Supplemental Materials
Expanded Materials and Methods Tables I–VII
Figures I–VII

REFERENCES
1. Bloem MW, Greenberg B, Jaarsma T, Januzzi JL, Lam CSP, Maggioni AP, Trochu JN, Butler J. Heart failure with reduced ejection fraction. Nat Rev Dis Primers. 2017;3:17058. doi: 10.1038/nrdp.2017.58
2. Reineke Y, Könnemann S, Chamling B, Gross S, Weitkamp K, Hoffmann W, Klingel K, Nauck M, Fleitze J, Dörö M, et al. Sugars make the difference - glycosylation of cardiodepressant antibodies regulates their activity in dilated cardiomyopathy. Int J Cardiol. 2019;292:156–159. doi: 10.1016/j.ijcard.2019.04.025
3. Deng W, Ednie AR, Qi J, Bennett ES. Averant siylation causes dilated cardiomyopathy and stress-induced heart failure. Basic Res Cardiol. 2016;111:57. doi: 10.1007/s00395-016-0574-1
4. Wen XY, Tarallo-Granacov M, Brand-Arzamendi K, Willems A, Rakic B, Huijbens K, Da Silva A, Pan X, El-Rass S, Ng R, et al. Sialic acid catabolism by N-acetyleneuraminic pyruvate lyase is essential for muscle function. JCI Insight. 2018;3:122573. doi: 10.1172/jci.insight.122573
5. Verdonchot JAJ, Wang P, Van Bilzen M, Haze broek MR, Merken JJ, Vanhoucke EK, Henkens MTHM, Van Den Wijngaard A, Glatz JFC, Krapels IPC, et al. Metabolic profiling associates with disease severity in nonischemic dilated cardiomyopathy. J Card Fail. 2020;26:212–222. doi: 10.1016/j.cardfail.2019.09.004
6. Jiang J, Pritesta N, Wang W, Zhang X, Wu Q; Effect of sialylated O-glycans in pro-brain natriuretic peptide stability. Clin Chem. 2010;56:995–996. doi: 10.1373/chlincem.2009.211738
7. Li F, Ding J. Sialylation is involved in cell fate decision during development, reprogramming and cancer progression. Protein Cell. 2019;10:550–565. doi: 10.1007/s13238-018-0957-9
8. Varki A; Sialic acids in human health and disease. Trends Mol Med. 2008;14:351–360. doi: 10.1016/j.molmed.2008.06.002
9. Haverkamp J, Schauer R, Wembter M; Neuraminidase derivatives newly discovered in humans: N-acetyl-9-O-L-fucosylneuraminic acid, N9-O-Diacetylneuraminic acid and N-acetyl-2,3-dehydro-2-deoxy neuraminic acid. Hoppe Seylers Z Physiol Chem. 1976;357:1699–1705. doi: 10.1515/bchm2.1976.357.1699
10. Contessotto P, Ellis BW, Jin C, Karlsson NG, Zorlutuna P, Kilcoyne M, Hoppe J, Krapels EK, Henkens MTHM, Van Den Wijngaard A, Glatz JFC, Krapels IPC, et al. Metabolic profiling associates with disease severity in nonischemic dilated cardiomyopathy. J Card Fail. 2020;26:212–222. doi: 10.1016/j.cardfail.2019.09.004
11. Wang SQ, et al. Glycans imaging in intact rat hearts and glycoproteomic analysis reveal the upregulation of sialylation during cardiac hypertrophy. J Am Chem Soc. 2014;136:17468–17476. doi: 10.1021/ja508484c
12. Varki A. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. Nature. 2007;446:1023–1029. doi: 10.1038/nature05816
13. Stocker RJ, Bennett ES. Differential sialylation modulates voltage-gated Na+ channel gating throughout the developing myocardium. J Gen Physiol. 2006;127:253–265. doi: 10.1085/jgp.200509423
14. Ufret-Vincenty CA, Baro DJ, Santana LF. Differential contribution of sialic acid to the function of repolarizing K+ currents in ventricular myocytes. Am J Physiol Cell Physiol. 2001;281:C464–C474. doi: 10.1152/ajpcell.2001.281.2.C464
15. Mandic R, Opper C, Krappe J, Wesemann W; Platelet sialic acid as a potential pathogenic factor in coronary heart disease. Thromb Res. 2002;106:137–141. doi: 10.1016/s0049-3848(02)00087-7
16. Sieve I, Rieche-Hoch M, Kasten M, Batta M, Stapel B, Falk CS, Leseegang MS, Haverich A, Scherr M, Hilfiker-Kleiner D. A positive feedback loop between IL-1β LPS and NEU1 may promote atherosclerosis by increasing pro-inflammatory state in monocytes and macrophages. Vascul Pharmacol. 2018;103:105–126. doi: 10.1016/j.vascpharm.2018.01.005
17. Zhang L, Wei TT, Li Y, Li J, Fan Y, Huang FQ, Cai YY, Ma G, Liu JF, Chen QQ, et al. Functional metabolomics characterizes a key role for N-acetyleneuraminic acid in coronary artery diseases. Circulation. 2018;137:1374–1390. doi: 10.1161/CIRCULATIONAHA.117.031139
18. Huang J, Li C, Song Y, Fan X, You L, Tan L, Xiao L, Li Q, Ruan G, Hu S, et al. ADRβ2 polymorphism Arg16Gly modifies the natural outcome of heart failure and dictates therapeutic response to β-blockers in patients with heart failure. Cells. 2018;7:528. doi: 10.3390/cells7060052
19. Hicks KA, Tcheng JE, Bokurz B, Chaitman BR, Cutlip DE, Farb A, Fonarow GC, Jacobs JP, Jaff MR, Lichtman JH, et al; American College of Cardiology; American Heart Association. 2014 ACC/AHA key data elements and definitions for cardiovascular endpoint events in clinical trials: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (Whiting Committee to Develop Cardiovascular Endpoints Data Standards). Circulation. 2015;132:302–361. doi: 10.1161/CIR.0000000000000156
20. Chang SC, Ren S, Rau CD, Wang JJ. Isoosylomeliduced heart failure: mouse model using osmic pump implantation. Methods Mol Biol. 2018;1816:207–220. doi: 10.1007/978-1-4939-8597-5_16
21. Yan M, Chen C, Gong W, Yin Z, Zhou L, Chaugi S, Wang DW; miR-21-3p regulates cardiac hypertrophic response by targeting histone deacetylase-8. Cardiovasc Res. 2015;105:340–352. doi: 10.1093/cvr/cvr254
22. Kostev K, Jacob L, Lucas A, Rathmann W; Low annual frequency of HbA1c testing in patients with Type 2 diabetes in primary care practices in Germany. Diabet Med. 2018;35:249–254. doi: 10.1111/dme.13556
23. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? *Int J Methods Psychiatr Res*. 2011;20:40–49. doi: 10.1002/mpr.329

24. Peng J, Vongpatanasin W, Sacharidou A, Kifer D, Yuhanna IS, Banerjee S, Tangaki K, Polasek O, Chu H, Sundgren NC, et al. Supplementation with the sialic acid precursor N-acetyl-D-mannosamine breaks the link between obesity and hypertension. *Circulation*. 2019;140:2005–2018. doi: 10.1161/CIRCULATIONAHA.119.034990

25. Glanz VY, Myasoedova VA, Grechko AV, Orekhov AN. Sialidase activity in human pathologies. *Eur J Pharmacol*. 2019;842:345–350. doi: 10.1016/j.ejphar.2018.11.014

26. Heimerl M, Sieve I, Ricke-Hoch M, Erschow S, Battmer K, Scherr M, Hilfiker-Kleiner D. Neuraminidase-1 promotes heart failure after ischemia/reperfusion injury by affecting cardiomyocytes and invading monocytes/macrophages. *Basic Res Cardiol*. 2020;115:62. doi: 10.1007/s00395-020-00821-z

27. Lindberg G, Råstam L, Gullberg B, Eklund GA. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow-up. *Int J Epidemiol*. 1992;21:253–257. doi: 10.1093/ije/21.2.253

28. Crook M, Lumb P, Andrews V, Swaminathan R. Serum total sialic acid, a reputed cardiovascular risk factor, and its relationship to lipids, plasma fasting insulin, blood pressure and body mass index in normal individuals. *Clin Sci (Lond)*. 1998;95:53–57. doi: 10.1042/cs0950053

29. Browning LM, Krebs JD, Jebb SA. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. *Metabolism*. 2004;53:899–903. doi: 10.1016/j.metabol.2004.01.013

30. Reganon E, Vila V, Martinez-Sales V, Vaya A, Lago A, Alonso P, Aznar J. Association between inflammation and hemostatic markers in atherothrombotic stroke. *Thromb Res*. 2003;112:217–221. doi: 10.1016/j.thromres.2003.12.008

31. von Itzstein M. The war against influenza: discovery and development of sialidase inhibitors. *Nat Rev Drug Discov*. 2007;6:967–974. doi: 10.1038/nrd2400

32. Glanz VY, Myasoedova VA, Grechko AV, Orekhov AN. Inhibition of sialidase activity as a therapeutic approach. *Drug Des Devel Ther*. 2018;12:3431–3437. doi: 10.2147/DDDT.S176220

33. Haxho F, Neufeld RJ, Szewczuk MR. Neuraminidase-1: a novel therapeutic target in multistage tumorigenesis. *Oncotarget*. 2016;7:40860–40881. doi: 10.18632/oncotarget.8396

34. Karhadkar TR, Pilling D, Cox N, Gomer RH. Sialidase inhibitors attenuate pulmonary fibrosis in a mouse model. *Sci Rep*. 2017;7:15069. doi: 10.1038/s41598-017-15198-8

35. Maisel A. Biomarkers in heart failure. Does prognostic utility translate to clinical futility? *J Am Coll Cardiol*. 2007;50:1061–1063. doi: 10.1016/j.jacc.2007.05.032

36. de Boer RA, Daniels LB, Maisel AS, Januzzi JL Jr. State of the Art: Newer biomarkers in heart failure. *Eur J Heart Fail*. 2015;17:559–569. doi: 10.1002/ejhf.273

37. Monti E, Bonten E, D'Azzo A, Bresciani R, Venerando B, Borsani G, Schauer R, Tettamanti G. Sialidases in vertebrates: a family of enzymes tailored for several cell functions. *Adv Carbohydr Chem Biochem*. 2010;64:3347–3356. doi: 10.1016/j.jcs.066966

38. Schmidt M, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja M, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet*. 1999;353:1649–1652. doi: 10.1016/s0140-6736(99)01046-6

39. Crook M, Lumb P, Andrews V, Swaminathan R. Serum total sialic acid, a reputed cardiovascular risk factor, and its relationship to lipids, plasma fasting insulin, blood pressure and body mass index in normal individuals. *Clin Sci (Lond)*. 1998;95:53–57. doi: 10.1042/cs0950053

40. Browning LM, Krebs JD, Jebb SA. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. *Metabolism*. 2004;53:899–903. doi: 10.1016/j.metabol.2004.01.013

41. Reganon E, Vila V, Martinez-Sales V, Vaya A, Lago A, Alonso P, Aznar J. Association between inflammation and hemostatic markers in atherothrombotic stroke. *Thromb Res*. 2003;112:217–221. doi: 10.1016/j.thromres.2003.12.008

42. von Itzstein M. The war against influenza: discovery and development of sialidase inhibitors. *Nat Rev Drug Discov*. 2007;6:967–974. doi: 10.1038/nrd2400

43. Glanz VY, Myasoedova VA, Grechko AV, Orekhov AN. Inhibition of sialidase activity as a therapeutic approach. *Drug Des Devel Ther*. 2018;12:3431–3437. doi: 10.2147/DDDT.S176220

44. Haxho F, Neufeld RJ, Szewczuk MR. Neuraminidase-1: a novel therapeutic target in multistage tumorigenesis. *Oncotarget*. 2016;7:40860–40881. doi: 10.18632/oncotarget.8396

45. Karhadkar TR, Pilling D, Cox N, Gomer RH. Sialidase inhibitors attenuate pulmonary fibrosis in a mouse model. *Sci Rep*. 2017;7:15069. doi: 10.1038/s41598-017-15198-8

46. de Boer RA, Daniels LB, Maisel AS, Januzzi JL Jr. State of the Art: Newer biomarkers in heart failure. *Eur J Heart Fail*. 2015;17:559–569. doi: 10.1002/ejhf.273

47. Monti E, Bonten E, D'Azzo A, Bresciani R, Venerando B, Borsani G, Schauer R, Tettamanti G. Sialidases in vertebrates: a family of enzymes tailored for several cell functions. *Adv Carbohydr Chem Biochem*. 2010;64:3347–3356. doi: 10.1016/j.jcs.066966

48. Schmidt M, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja M, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet*. 1999;353:1649–1652. doi: 10.1016/s0140-6736(99)01046-6