Additional File 1

Contents for Additional File 1:

Additional File 1

Data Collection and Processing

Data extraction was executed using PostgreSQL and Navicat Premium with Structured Query Language (SQL). The extracted variables were categorized into seven main groups: **Demographics** (age, gender, race); **Vital Signs** (systolic blood pressure [SBP], diastolic blood pressure [DBP], heart rate [HR], respiratory rate [RR], first Glasgow Coma Scale [GCS]); **Comorbidities** (including hypertension, diabetes, atrial fibrillation [AF], heart failure [HF], coronary heart disease [CHD], chronic obstructive pulmonary disease [COPD], respiratory failure [RF], chronic kidney disease [CKD], kidney failure [KF], anemia, sepsis, and tumor); **Severity Scores** (Acute Physiology Score III [APSIII], Oxford Acute Severity of Illness Score [OASIS], Simplified Acute Physiology Score II [SAPSII], Sequential Organ Failure Assessment score at 1 hour [SOFA-1h], Systemic Inflammatory Response Syndrome [SIRS], Charlson Comorbidity Index [CCI]); **Laboratory Parameters** (Red Blood Cell count [RBC], White Blood Cell count [WBC], Platelet count, Mean Glucose [Glu Mean], Prothrombin Time [PT], Activated Partial Thromboplastin Time [APTT], Anion gap, Bicarbonate, Blood Urea Nitrogen [BUN], Sodium, Potassium, Calcium, Chloride, Creatinine, Hemoglobin, Mean Corpuscular Hemoglobin [MCH], Mean Corpuscular Hemoglobin Concentration [MCHC], International Normalized Ratio [INR]); **Medical History** (antidiabetic and antithrombotic medications); **Outcomes** (poor outcomes at discharge, 90-day and 1-year mortality and survival time, ICU and hospital LOS).

All baseline characteristics, excluding Glycemic Variability (GV), were derived from data collected within the first 24 hours of ICU admission. If multiple recordings occurred, the first value was used. The follow-up period started at admission and extended to one year post-discharge. GV was calculated as the ratio of standard deviation (SD) to mean of all glucose measurements during ICU stays, with frequency and timing tailored to patient needs. Glucose data collected before outcomes were included to reflect real-world monitoring. To analyze the relationship between GV and clinical outcomes, patients were stratified into four groups based on GV quartiles (Q1–Q4). The quartiles were defined as follows: Q1 (lowest GV), representing the 1st quartile (<25th percentile); Q2 (moderate-low GV), encompassing the 25th–50th percentile; Q3 (moderate-high GV), covering the 50th–75th percentile; and Q4 (highest GV), defined as the 75th percentile and above, as described in prior studies. This stratification method aligns with established approaches in previous research. This approach enabled a comprehensive assessment of the relationship between GV and clinical outcomes, providing greater insight into the impact of glucose variability.

To mitigate bias, we used the random forest algorithm to impute variables with less than 20% missing data. **Additional File 1: Figure S1** visualized missing data patterns, with the y-axis showing variables and the x-axis indicating the percentage of

missing data. Color bands denote missing data extent: green (<5%) and yellow (5%-20%).

The Specific Variables Included in the Final Model

The variables included in the final model were: Gender, Age, Race, SBP, DBP, HR, RR, Hypertension, Diabetes, AF, Copd, Spesis, Tumor, Antidiabetic, Antithrombotic, WBC, Platelet, APTT, Sodium, Potassium, MCHC, INR.

Statistical Analyses

Baseline characteristics were summarized using descriptive statistics. Continuous variables are expressed as mean (SD) or median (interquartile range [IQR]), with comparisons made using t-test or nonparametric tests as appropriate. Categorical variables are presented as frequencies and percentages, with comparisons conducted using Pearson's chi-square or Fisher's exact tests.

Survival Analysis: Kaplan-Meier analysis evaluated cumulative mortality and survival probabilities at 90 days and 1 year across GV-stratified groups, with log-rank tests used for group differences.

Regression Analyses: Multiple regression approaches were used to examine associations between GV and outcomes. Logistic regression estimated ORs and 95% confidence intervals (CIs) for GV and poor discharge outcomes. Cox models calculated hazard ratios (HRs) and 95% CIs for GV with 90-day and 1-year mortality. Linear regression estimated beta coefficients for GV and LOS. All analyses were adjusted for potential confounders in three models, with variable selection informed by clinical relevance and prior evidence of their association with glycemic variability and clinical outcomes in critically ill patients [1–6]. The models were designed to comprehensively adjust for confounding while avoiding overfitting: Model 1 (unadjusted), Model 2 (adjusted for gender, age, and race), and Model 3 (adjusted for gender, age, race, SBP, DBP, HR, RR, hypertension, diabetes, AF, COPD, sepsis, tumor, antidiabetic medication use, antithrombotic medication use, WBC, platelet count, APTT, sodium, potassium, MCHC, and INR). The specific variables included in the final model are detailed in **Additional File 1**. GV was analyzed as both a continuous variable and by quartiles, with the lowest quartile as reference. No substantial multicollinearity was detected (VIF < 2 for all variables; **Additional file 2: Tables S2-S7**).

Non-linear Relationships: Restricted cubic splines (RCSs) with four knots (5th, 35th, 65th, and 95th percentiles) explored non-linear GV-outcome relationships. Wald tests assessed non-linearity. Inflection points and adjusted HRs were calculated to identify thresholds and quantify risks.

Predictive Ability: Area Under Curve (AUC), net reclassification improvement (NRI), and integrated discrimination improvement (IDI) evaluated the predictive

ability of incorporating GV into scoring tools. NRI is used to assess the improvement in correct patient classification of a new model compared to the original model [7]. It considers changes in classification, including the proportion of correct upward reclassification (high risk) and correct downward reclassification (low risk). IDI, on the other hand, measures the improvement in predictive probability of the new model, reflecting the magnitude of change in predicted probabilities [8]. Together, these two metrics provide a comprehensive evaluation of the enhancement in a model's predictive capability.

Robustness Testing: The effect of GV on outcomes was examined using four missing data interpolation methods: K-Nearest Neighbors (KNN), median imputation, regression imputation, and exclusion of missing data. Subgroup analyses were conducted for age (<65 or ≥65 years), gender, hypertension, diabetes, and AF using the four imputation methods. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using R version 4.2.2.

Survival Analysis

Kaplan-Meier analysis showed significant differences in cumulative mortality between high (Q3+Q4) and low (Q1+Q2) GV groups at 90-day and 1-year. Higher GV groups had substantially higher cumulative mortality compared to lower groups (**Additional File 1: Figure S2**). Log-rank tests indicated significant differences between mortality curves ($P< 0.05$) for both time points and conditions.

Kaplan-Meier survival analysis by GV quartiles at 90-day and 1-year showed a clear association between GV and mortality risk. In **Figures 3**, the highest GV quartile (Q4) consistently showed the lowest survival probability, followed by Q3, Q2, and Q1. The separation between curves was apparent early and became more pronounced over time, indicating both immediate and persistent impacts of GV on mortality risk.

Predictive Capacity and Incremental Value of GV

To evaluate the predictive capacity of GV for poor outcomes at discharge, 90-day, and 1-year mortality, we calculated the AUC (**Additional File 1: Figure S4 and Additional File 2: Table S12**). GV had an AUC of approximately 0.59 for predicting poor discharge outcomes (IQR: 0.590 [0.537, 0.643], numeric: 0.595 [0.542, 0.648]) with a cut-point of 0.126. The AUC for predicting 90-day mortality was around 0.58 (IQR: 0.578 [0.534, 0.622], numeric: 0.586 [0.540, 0.631]) with a cut-point of 0.206. For 1-year mortality, GV's AUC was about 0.56 (IQR: 0.565 [0.523, 0.606], numeric: 0.566 [0.522, 0.609]) with a cut-point of 0.206.

Additional File 2: Table S13 shows the incremental effect of GV on scoring tools (APSIII, OASIS, SAPSII, SOFA 1h, SIRS, and CCI) for predicting poor

outcomes at discharge and mortality. NRI and IDI were used to assess GV's impact. NRI analysis showed that adding categorical GV significantly improved predictive performance for all tools and outcomes, except APSIII for 1-year mortality (P<0.05). Numeric GV also improved predictions significantly for most tools and outcomes. IDI analysis showed significant improvements for SAPSII, SOFA 1h, and SIRS in predicting poor discharge outcomes when GV was added.

Sensitivity Analyses

Additional File 2: Tables S14-S21 provided detailed results of four sensitivity analyses for Model 3, Q4 (highest quartile of GV) in relation to poor discharge outcomes, 90-day and 1-year mortality, and GV's impact on ICU/hospital LOS. These analyses, using KNN, median imputation, regression imputation, and exclusion of missing data, showed consistent associations (**Figure 6**). GV was significantly associated with poor discharge outcomes, 90-day, and 1-year mortality across all methods. Adjusted ORs and HRs consistently exceeded unity, aligning with **Table 2** results derived using random forest imputation.

Sensitivity analyses on GV's relationship with ICU and hospital LOS were conducted. In **Figure 6E**, examining GV and hospital LOS in the hemorrhage cohort, the lower bound of the confidence interval of KNN, regression imputation, and exclusion of missing data methods is negative, consistent with random forest results (**Additional File 2: Table S10**). To further validate robustness, sensitivity analyses on subgroups were performed. **Additional File 1: Figures S5-S8** illustrate consistent ORs, HRs, and beta coefficients across all methods. This consistency across approaches to missing data interpolation substantiates the robustness of GV's relationship with poor outcomes at discharge, enhancing the reliability and generalizability of our findings, and supporting the clinical significance of GV in these populations.

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Additional File 1

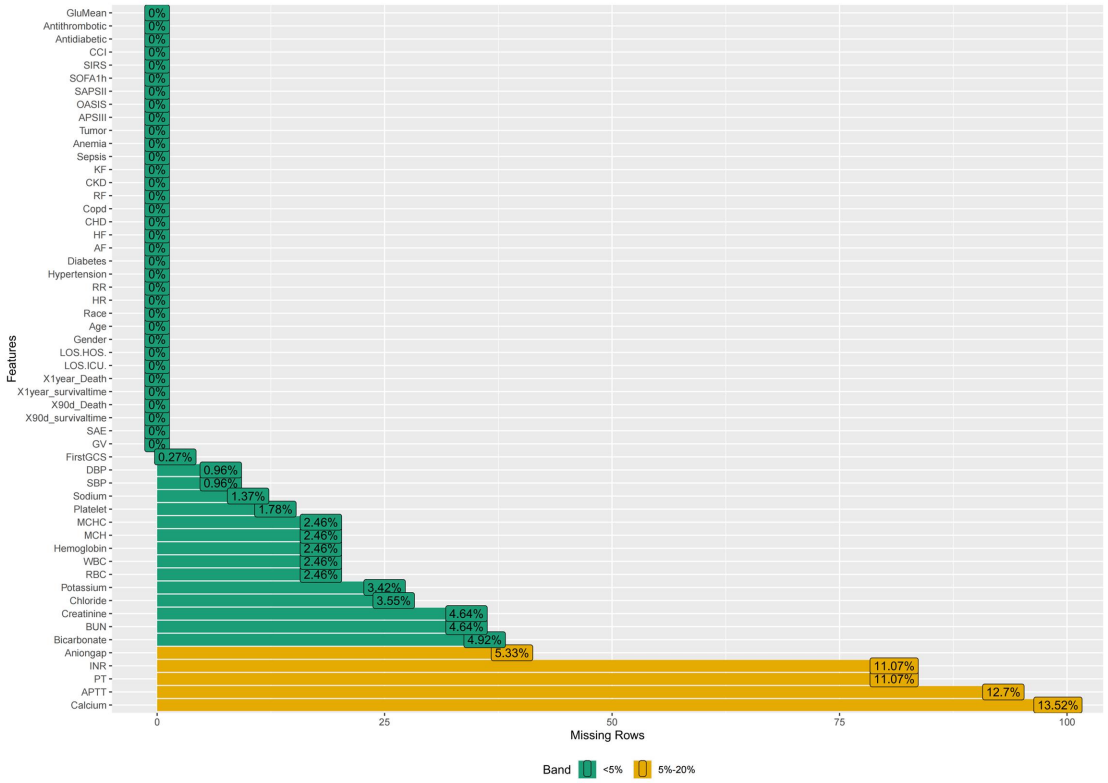

Figure S1. Missing Data Visualization for Non-Traumatic Cerebral Hemorrhage Patients.

SAE (Serious Adverse Event) is defined as poor outcomes at discharge in this study.

Abbreviations: APTT, Activated Partial Thromboplastin Time; PT, Prothrombin Time; INR, International Normalized Ratio; BUN, Blood Urea Nitrogen; RBC, Red Blood Cell; WBC, White Blood Cell; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; GCS, Glasgow Coma Scale; GV, Glycemic Variability; ICU, Intensive Care Unit; LOS, Length of Stay; HR, Heart Rate; RR, Respiratory Rate; AF, Atrial Fibrillation; HF, Heart Failure; CHD, Coronary Heart Disease; COPD, Chronic Obstructive Pulmonary Disease; RF, Respiratory Failure; CKD, Chronic Kidney Disease; KF, Kidney Failure; APSIII, Acute Physiology Score III; OASIS, Oxford Acute Severity of Illness Score; SAPSII, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; SIRS, Systemic Inflammatory Response Syndrome; CCI, Charlson Comorbidity Index; GluMean, Mean Glucose Levels.

Additional File 1

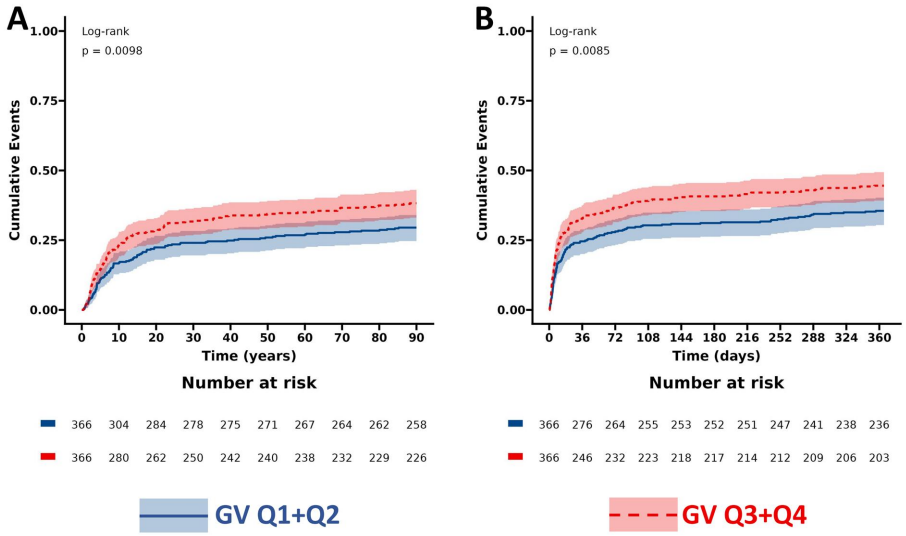

Figure S2. Kaplan–Meier Survival Analysis Curves of Cumulative Mortality of Patients for 90-day and 1-year Mortality by High and Low GV Quartiles.

Kaplan–Meier curves illustrating the cumulative probability of mortality for patients stratified by high (Q3+Q4) and low (Q1+Q2) GV quartiles: (A) 90-day mortality; (B) 1-year mortality.

GV Quartile Ranges: Q1: [0.0045, 0.105); Q2: [0.105, 0.15); Q3: [0.15, 0.215); Q4: [0.215, 1.3]

Abbreviation: GV, Glycemic Variability.

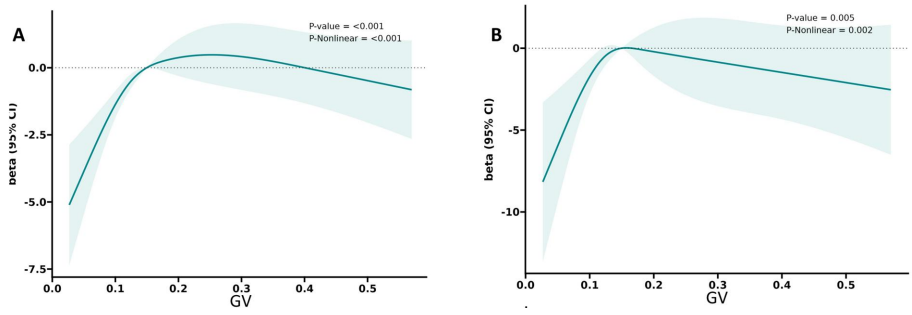

Figure S3. RCS Curves for the Relationship Between GV and Length of Stay in ICU/Hospital.

Models adjusted for: Gender, Age, Race, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart Rate (HR), Respiratory Rate (RR), Hypertension, Diabetes, Atrial Fibrillation (AF), Chronic Obstructive Pulmonary Disease (COPD), Sepsis, Tumor, Antidiabetic medication, Antithrombotic medication, White Blood Cell count (WBC), Platelet count, Activated Partial Thromboplastin Time (APTT), Sodium, Potassium, Mean Corpuscular Hemoglobin Concentration (MCHC), and International Normalized Ratio (INR).

RCS Curves of GV and Beta Coefficients: (A) RCS curve for Length of Stay in ICU;

(B) RCS curve for Length of Stay in Hospital.

Abbreviation: RCS, Restricted Cubic Spline; GV, Glucose Variability; ICU, Intensive Care Unit.

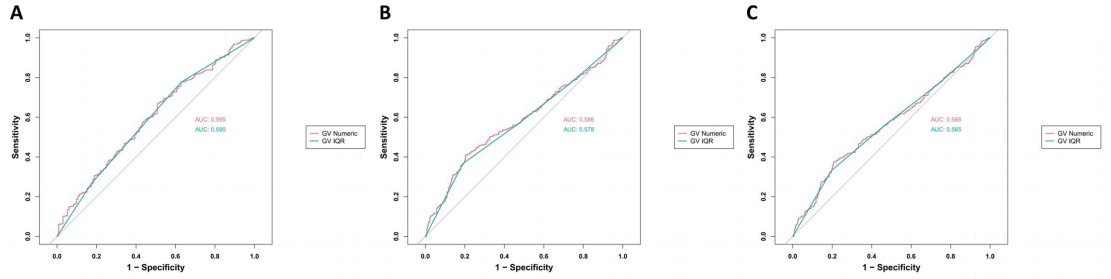

Figure S4. ROC Curve Analysis of Glycemic Variability Predicting Outcomes in Patients.

(A) ROC curve analysis of GV predicting Poor Outcomes at Discharge;

(B) ROC curve analysis of GV predicting 90-day mortality;

(C) ROC curve analysis of GV predicting 1-year mortality;

Abbreviations: GV, Glycemic Variability; AUC, Area Under the Curve; ROC, Receiver Operating Characteristic.

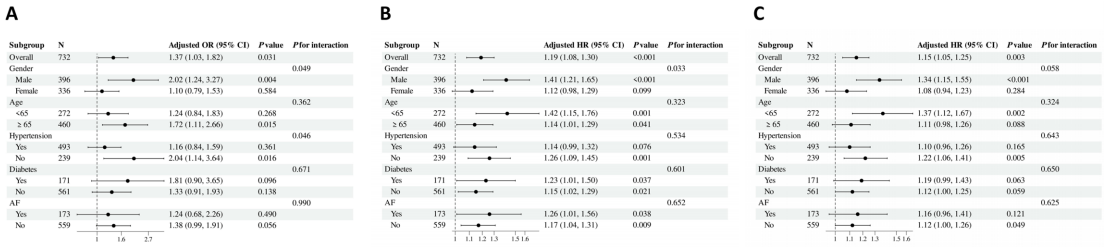

Figure S5. Forest Plot of the Association Between Glucose Variability and Clinical Outcomes Using KNN Data Interpolation Method.

(A) Association between GV and Poor Outcomes at Discharge;

(B) Association between GV and 90-day mortality;

(C) Association between GV and 1-year mortality;

Abbreviations: GV, Glycemic Variability; HR, hazard ratio; CI, confidence interval; BMI, body mass index; AF, atrial fibrillation; KNN, K-Nearest Neighbors.

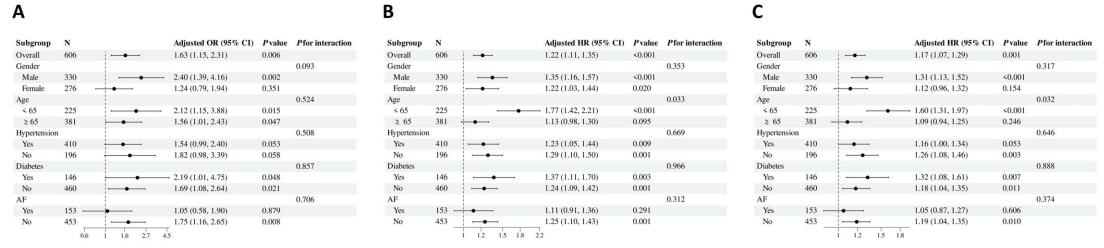

Figure S6. Forest Plot of the Association Between Glucose Variability and Clinical Outcomes Using Median Data Interpolation Method.

(A) Association between GV and Poor Outcomes at Discharge;

(B) Association between GV and 90-day mortality;

(C) Association between GV and 1-year mortality;

Abbreviations: GV, Glycemic Variability; HR, hazard ratio; CI, confidence interval; BMI, body mass index; AF, atrial fibrillation.

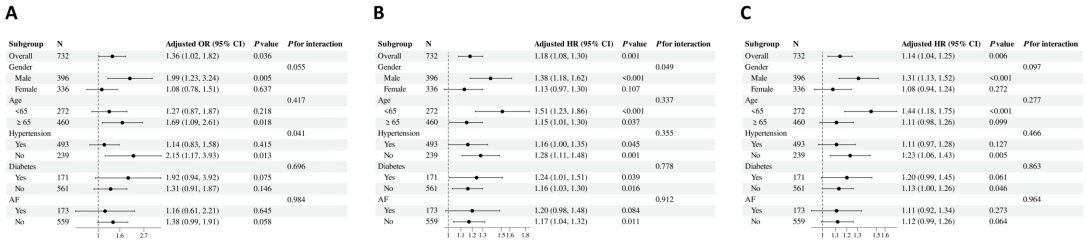

Figure S7. Forest Plot of the Association Between Glucose Variability and Clinical Outcomes Using Regression Data Interpolation Method.

(A) Association between GV and Poor Outcomes at Discharge;

(B) Association between GV and 90-day mortality;

(C) Association between GV and 1-year mortality;

Abbreviations: GV, Glycemic Variability; HR, hazard ratio; CI, confidence interval; BMI, body mass index; AF, atrial fibrillation.

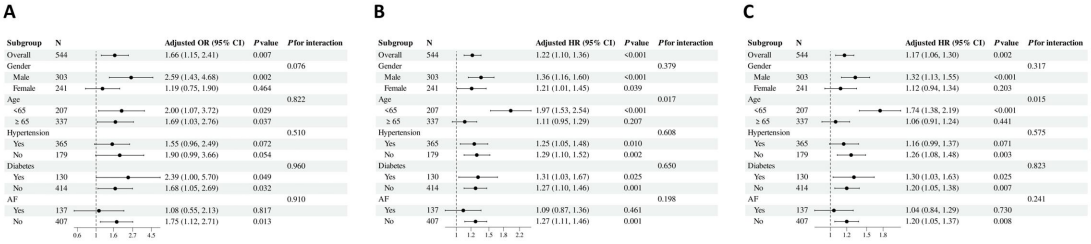

Figure S8. Forest Plot of the Association Between Glucose Variability and Clinical Outcomes Using Excluding Missing Data Interpolation Method.

(A) Association between GV and Poor Outcomes at Discharge;

(B) Association between GV and 90-day mortality;

(C) Association between GV and 1-year mortality.

Abbreviations: GV, Glycemic Variability; HR, hazard ratio; CI, confidence interval; BMI, body mass index; AF, atrial fibrillation.

Additional File 1

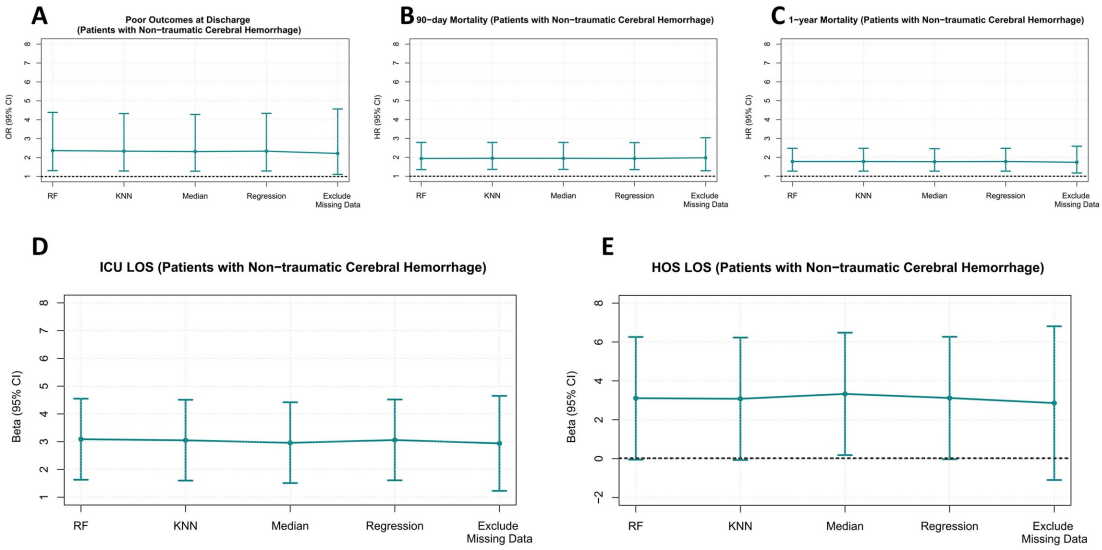

Figure S9. Sensitivity Analyses of Glycemic Variability on Clinical Outcomes Using Different Methods of Missing Data Interpolation in Model 3.

Model adjusted for: Gender, Age, Race, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart Rate (HR), Respiratory Rate (RR), Hypertension, Diabetes, Atrial Fibrillation (AF), Chronic Obstructive Pulmonary Disease (COPD), Sepsis, Tumor, Antidiabetic medication, Antithrombotic medication, White Blood Cell count (WBC), Platelet count, Activated Partial Thromboplastin Time (APTT), Sodium, Potassium, Mean Corpuscular Hemoglobin Concentration (MCHC), International Normalized Ratio (INR).

(A-C) Impact of GV on Poor Outcomes at Discharge 90-day mortality, and 1-year mortality;

(G-H) Impact of GV on Length of Stay in ICU and Hospital.

Abbreviations: GV, Glycemic Variability; ICU, Intensive Care Unit; LOS, Length of Stay; RF, Random Forest; KNN, K-Nearest Neighbors.