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# J-shaped associations and joint effects of fasting glucose with inflammation and cytokines on COVID-19 mortality

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ABSTRACT

*Objectives:* The aim of this study was to investigate the dose-response relationship of admission fasting glucose (FBG) with corona virus disease 2019 (COVID-19) mortality and to further evaluate potential interactions of hyperglycemia with inflammation and hypercoagulation on COVID-19 outcomes.
*Methods:* This retrospective study included 2555 consecutively hospitalized patients with COVID-19, until death or discharge, in Wuhan Union hospital between January 1 and April 9, 2020. The poor early outcomes included admission to intensive care unit, intubation, and deaths occurring within 28 days. We used splines nested in Cox regression to visualize dose-response associations and generalized additive models to fit three-dimensional (3D) trend plots for joint effects of FBG with markers of inflammation and coagulation.
*Results:* J-shaped associations existed between hospitalized mortality or poor early outcomes and FBG with a nadir at 5 mmol/L, which were more evident in women. 3D plots demonstrated significant joint effect trends, and patients with hyperglycemia and high neutrophil-lymphocyte ratio, C-reactive protein, lactate dehydrogenase, procalcitonin, d-dimer, and interleukin-6 had 7.4-25.3-fold risks; the proportions of joint associations attributed to additive interactions reached 30% to 54%.
*Conclusions:* FBG was associated with hospitalized mortality and poor early outcomes in a J-shaped manner, and a combination of hyperglycemia, inflammation, hypercoagulation, and cytokines conferred a dramatically higher risk.



## Introduction

A large number of studies have shown that diabetes mellitus and hyperglycemia are linked to increased mortality and poor early outcomes in patients with coronavirus disease 2019 (COVID-19) (Barron et al., 2020; Carrasco-Sánchez et al., 2021; Ciardullo et al., 2021; Coppelli et al., 2020; Klonoff et al., 2021; Lazarus et al., 2021; Seiglie et al., 2020; Tornhammar et al., 2021; Wang et al., 2020; Wu et al., 2021; Yang et al., 2021; Zhu et al., 2020). However, uncertainties remained in the linearity, shape, magnitude, and consistency of the associations. Most previous studies had limitations in small samples and insufficient power (Fox et al., 2021; Huang et al., 2020; Li et al., 2020; Saand et al., 2021; Zhang et al., 2020), and few of them have further explored the potential linear or nonlinear dose-response associations (Alahmad et al., 2020; Shen et al., 2021; Zhu et al., 2020). An earlier study found a J-shaped association between fasting blood glucose (FBG) and COVID-19 severity in 293 Chinese patients without pre-existing diabetes mellitus (Zhu et al., 2020), whereas another study conducted in 417

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Kuwaiti patients showed a key nonlinear trend with a steeper slope that became flat when FBG levels reached approximately 10 mmol/L(Alahmad et al., 2020). Very recently, a cross-sectional study in 35 inpatients found a U-shaped curve between mean glucose levels and composite adverse outcomes (Shen et al., 2021).

On the other hand, patients with diabetes mellitus have increasing levels of chronic inflammation owing to underlying insulin resistance and hyperglycemia (Rajpal et al., 2020). In addition, it was reported that the immune response, inflammation, coagulation, and cytokine profiles increased in patients with COVID-19 with diabetes mellitus (Dhar et al., 2021; Lim et al., 2021; Zhao et al., 2020; Zheng et al., 2021). Vasbinder et al. found that individuals with diabetes and hyperinflammation assessed by soluble urokinase plasminogen activator receptor (suPAR) had a 53.8% incidence of composite endpoint (including in-hospital death, need for mechanical ventilation, and need for renal replacement therapy), compared with 23.9% in individuals with low-level suPAR (Vasbinder et al., 2022). We thus hypothesized that the exacerbation of pre-existing chronic inflammation with hyperglycemia might enhance intense hyperimmune response to the infection ("cytokine storm") and hyperinflammation, and show strong joint effects and play a critical role in mortality and poor early outcomes of COVID-19 (Lim et al., 2021; Rajpal et al., 2020). As a result, we might have underestimated the role of hyperglycemia in mortality of patients with COVID-19. However, to the best of our knowledge, no previous study has evaluated the potential additive interactions and joint effects of hyperglycemia with inflammation biomarkers, coagulation biomarkers, and cytokines on mortality and poor early outcomes.

The present study aimed to investigate the dose-response associations of FBG with hospitalized mortality and poor early outcomes, and further explore the potential joint effects of FBG with inflammation, coagulation, and cytokines.

## Subjects, Materials and Methods

### *Study design and population*

This large retrospective cohort study enrolled 2691 diagnosed adult patients (≥18 years) with COVID-19, according to the WHO interim guidance, who were admitted to Wuhan Union Hospital (including the general hospital, west court hospital and tumor center hospital) and Wuhan Red Cross Hospital from January 1 to April 9, 2020. All patients were laboratory-confirmed by real-time reverse-transcription polymerase chain reaction assay from throat swab specimens and were followed up until death or discharge, or until the final date of April 9th was reached. Excluded from the analysis were 83 patients without FBG, 37 patients who died within 24 hours after admission, and 16 patients who died after 28 days of continuous hospitalization. Particularly, all the excluded patients also covered 2 patients who were treated with steroids for Behcet's disease and scleroderma before admission, which may have potential confounding effect. As a result, a total of 2555 patients were included in the present study.

This study was approved by the ethical committee of Wuhan Red Cross hospital and Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was waived by the Ethics Committee owing to emerging infectious disease and the retrospective and anonymous nature of the study.

### *Data collection*

Demographic (age, sex), medical history (comorbidities and medication history), clinical (symptoms and hospitalization treatment), laboratory examination, and outcomes data were obtained from electronic medical records using a standardized data collection form modified based on the World Health Organization/International Severe Acute Respiratory and Emerging Infection Consortium case record forms. All the data were reviewed independently by two first-line physicians (Shan Deng and Ru Chen) and were further double-checked by other researchers (Lizhi Hu, Min Chen, and Minglu Liang). Comorbidities included chronic pulmonary disease, hypertension, diabetes mellitus, cardiovascular disease, cerebrovascular disease, chronic kidney disease, and malignancy. Laboratory tests were conducted through standard morning fasting blood biochemistry. We collected data on complete blood count including neutrophil-lymphocyte ratio (NLR), coagulation profiles (d-dimer, fibrinogen), serum biochemical tests such as FBG, blood lipids, liver and renal function, C-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin (PCT), and cytokines such as interleukin-6 (IL-6). In particular, estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI equation (Levey et al., 2009).

### *Definition and outcomes*

The discharge criteria were temperature normalization for over three days, relief of clinical symptoms, substantial improvement in imaging of both lungs, and throat-swab samples returning negative twice at least 24 hours apart. All FBG values were measured after overnight fasting on the morning of the second day after admission and were also the first available values during hospitalization. The outcomes of interest were all-cause hospitalized mortality and poor early outcomes, including intubation, admission to intensive care unit (ICU), or death, which were confirmed through medical records and death certification. All the above outcomes were defined as events occurring beyond 24 hours after admission but within 28 days of continuous hospitalization. The time to events was calculated as the days from admission to death, composite end points, or discharge. In particular, the follow-up time for poor early outcomes was defined as the time of intubation, admission to ICU, or death, whichever came first.

### *Statistical analysis*

Continuous variables were expressed as median (the first and third IQR), and categorical variables were described as counts (%) of participants. Baseline characteristics across survivors and dead patients were compared using *t*-test or Mann-Whitney U test, depending on the distribution of continuous variables, and chi-square tests were used for category variables. Based on the definition of hypoglycemia for diabetes mellitus patients and the latest Standards of Medical Care in Diabetes from the American Diabetes Association, FBG was classified into five categories: <4.0 mmol/L, 4.0-6.1 mmol/L, 6.1-7.8 mmol/L, 7.8-10.0 mmol/L, and >10.0 mmol/L(American Diabetes, 2020). Baseline comorbidities across these five categories of FBG were also compared with the analysis of variance and chi-square test. Cox proportional hazard regressions were used to evaluate the relationship of FBG categories with mortality and poor early outcomes after adjustment for major covariates, with the second group (the lowest incidence of outcomes) as reference. Confounders were determined according to previous studies and outcomes of univariate analysis. Finally, models were adjusted for age, sex, comorbidities (chronic pulmonary disease, hypertension, diabetes mellitus, cardiovascular disease, chronic kidney disease, and malignancy), total cholesterol, triglyceride, alanine transaminase (ALT), aspartate aminotransferase (AST), eGFR, NLR, hematocrit, activated partial thromboplastin time (aPTT), antibiotics treatment, and corticosteroids treatment. To further explore whether the associations were independent of other inflammation and procoagulation parameters, we

also conducted additional sensitivity analysis by further adjusting for CRP, LDH, and d-dimer, even though it may have been overfitted. Furthermore, FBG as both linear and quadratic terms was simultaneously entered in the models to test the nonlinear relationship. We further examined the nonlinear relationship by plotting restricted cubic splines using 5 knots placed at fifth, 25th, 50th, 75th, and 95th percentiles of FBG levels, with 7.0 mmol/L as the reference. Stratified analyses were performed according to age (<65 years, ≥65 years) and sex (male, female). The multiplicative interaction tests between FBG levels and each stratification factor were conducted by adding a cross-product in the models. The restricted cubic splines were also plotted respectively according to stratification factors. Then, we adopted generalized additive models to fit 3D trend plots to further visualize the joint effects of continuous FBG levels with inflammation markers (NLR; CRP; LDH; PCT), coagulation markers (d-dimer), and cytokines (IL-6). Moreover, we dichotomized FBG (<7.0 mmol/L, ≥7.0 mmol/L) base on the clinical reference range, and dichotomized NLR (<5.00, ≥5.00), LDH (<294 U/L, ≥294 U/L), PCT (<0.13 ng/mL, ≥0.13 ng/mL), d-dimer (<1.3 mg/L, ≥ 1.3 mg/L), IL-6 (<19.0 ng/mL, ≥19.0 ng/mL) by the 75th percentile as either low or high groups. To keep a relatively large sample size and sufficient power in each group, we dichotomized CRP as low or high groups according to 30.0 mg/L. We further quantitatively calculated the joint effects of hyperglycemia with the above high groups of inflammation, coagulation, and cytokines, respectively, with normoglycemia and the corresponding low groups as reference. Meanwhile, the relative excess risk due to interaction (RERI) of the joint effects and the attributable proportion due to additive interactions (AP) were also calculated.

All analyses were performed using SAS software (version 9.4; SAS Institute Inc, Cary, NC) and R software (version 4.0.3; the R foundation). All *P* values for the tests were two-sided, and *P* values < 0.05 were considered as statistically significant.

## Results

### *Characteristics of study population*

As shown in Table 1, of the 2555 included patients, the mean age (the first and third IQR) was 60.0 years (48.0-68.0), and 1222 patients (47.8%) were men. Two hundred twelve patients (8.3 %) died in hospitals, and 241 (9.4 %) had poor early outcomes. Patients who died in hospitals had an average age of 69.0 years. They were mostly men and more likely to have chronic comorbidities. The complete blood count showed that dead patients had higher white blood cell, neutrophil count, and NLR, but lower lymphocyte count and blood platelets, although there was no significant difference in red blood cell, hematocrit, and hemoglobin. The coagulation markers (e.g., aPTT and d-dimer), inflammatory markers (e.g., CRP, LDH and procalcitonin), and cytokines (e.g., IL-6 and IL-10) of the dead patients were higher than those of the survivors. In addition, FBG, cholesterol, and liver and renal function parameters such as ALT, AST, and CR were high among dead patients. In contrast, eGFR was relatively lower. In particular, the dead patients had rather high proportions of treatments with high-flow nasal cannula oxygen, noninvasive ventilation, and corticosteroid treatment. In addition, compared with the optimal glucose management target (4.0 ≤ FBG < 6.1 mmol/L), as expected, hypertension, diabetes, cardiovascular disease, and chronic kidney disease were more common among patients with hypoglycemia and hyperglycemia, except for chronic pulmonary disease and malignancy (Table S1).

### *Relationships of FBG with mortality and poor early outcomes*

Compared with the optimal glucose management target (4.0 ≤ FBG < 6.1 mmol/L), patients with hypoglycemia or hyperglycemia had higher risk of mortality and poor early outcomes, which showed a J-shaped dose-response relationship (Table 2). The fully adjusted HRs (95% CI) for hospitalized mortality across the five categories of FBG levels were 3.13 (1.07, 9.19), 1.00 (reference), 2.52 (1.64, 3.89), 2.59 (1.57, 4.27), and 4.44 (2.59, 7.62), respectively (*P* for quadratic trend=0.01). Similarly, the fully adjusted HRs (95% CI) of poor early outcomes were 2.09 (0.71, 6.72), 1.00 (reference), 2.32 (1.57, 3.45), 2.05 (1.28, 3.30), and 3.72 (2.25, 6.15), respectively (*P* for quadratic trend=0.059). Besides, the sensitivity analysis results also confirmed that hyperglycemia (FBG>10 mmol/L) was associated with in-hospital mortality and poor early outcomes, independent of inflammation and procoagulation. However, the increased risk in patients with hypoglycemia was not significant owing to more missing data and insufficient power (Table S2).

Furthermore, the restricted cubic splines clearly showed J-shaped curve associations of continuous FBG levels with risk for mortality and poor early composite outcomes with a nadir at approximately 5 mmol/L (Figure 1). It was particularly worth noticing that FBG levels above 5 mmol/L were linearly associated with increased risk of mortality and poor early composite outcomes. In addition, the association between FBG and mortality seemed to be more evident in patients younger than 65 years than in the counterparts (*P* for interaction = 0.054). In particular, the associations of FBG with mortality and poor early outcomes were stronger in women than in men (*P* for interactions < 0.001). When FBG was above 5 mmol/L, the curve slope was clearly steeper in women than in men. For instance, compared with patients with FBG at 7.0 mmol/L, those with FBG at 11.0 mmol/L had 1.81-fold mortality risk (HR: 1.81; 95% CI: 1.26, 2.60) in men and 2.97-fold risk (HR: 2.97; 95% CI: 1.87, 4.72) in women. Similarly, there were 1.71-fold risk (HR: 1.71; 95% CI: 1.22, 2.40) in men and 2.6-fold risk (HR: 2.55; 95% CI: 1.67, 3.91) in women for poor early outcomes.

### *Additive interactions and joint effects of FBG with inflammation, coagulation, and cytokines*

The joint effect trends were clearly shown by the 3D plots (Figure 2). The risk surfaces for all the subgraphs showed a steeper uptrend with the joint increase of FBG with markers of inflammation and coagulation and cytokines. Hyperglycemia with high levels of NLR, CRP, LDH, PCT, d-dimer, and IL-6 had greater joint effects on risk of hospitalized mortality and poor early outcomes. In particular, the joint effect trends of FBG with NLR, CRP, and LDH seemed obviously stronger.

As shown in Figure 3, compared with patients with normoglycemia and low levels of inflammation, coagulation, and cytokines, the increased HRs (95% CI) of mortality in patients with hyperglycemia and high levels of NLR, CRP, LDH, PCT, d-dimer, and IL-6 were 16.07 (9.50, 27.18), 25.31 (12.14, 52.80), 16.46 (9.66, 28.02), 11.51 (5.79, 22.89), 7.39 (4.91, 11.12), and 25.09 (8.37, 75.17), respectively. Correspondingly, regarding the additive interactions, the RERI (HR; 95% CI) of mortality was 4.80 (0.15, 9.46), 8.22 (–0.26, 16.71), 8.30 (2.78, 13.81), 4.67 (0.20, 9.13), 3.32 (1.15, 5.49), and 13.53 (–3.85, 30.91), respectively. In addition, the AP (HR; 95% CI) was 30% (8%, 52%), 32% (11%, 54%), 50% (33%, 67%), 41% (15%, 66%), 45% (24%, 65%), and 54% (26%, 81%), respectively. Meanwhile, similar but weaker additive interactions and joint effects were observed for poor early outcomes.

## Discussion

Our large retrospective study demonstrated a J-shaped association of admission FBG levels with increased risk of hospitalized death and poor early composite outcomes in patients with COVID-19 with a nadir at 5 mmol/L of FBG, which was more prominent

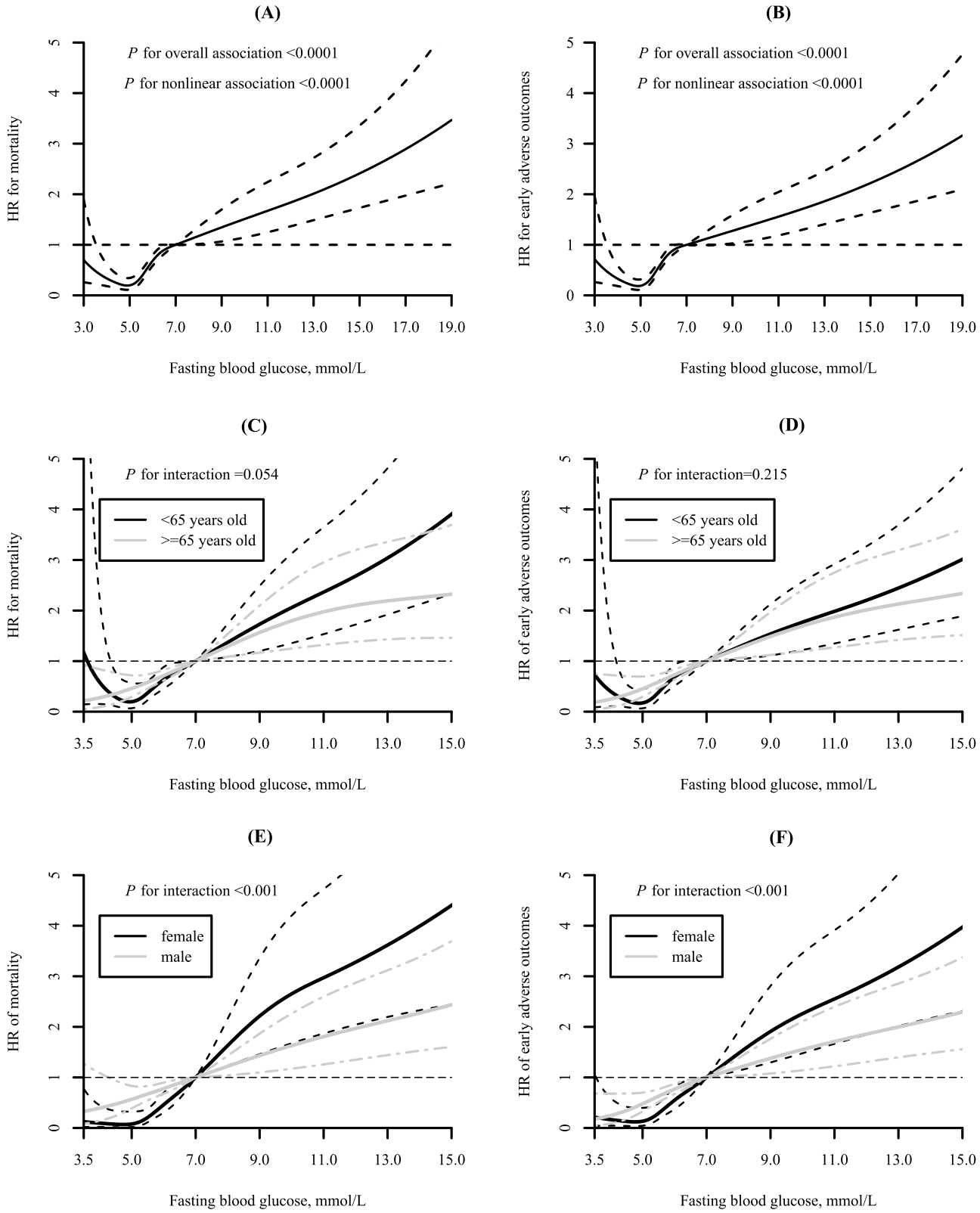


**Figure 1.** The restricted cubic splines for multivariable-adjusted dose-response associations of fasting blood glucose at admission with risk for hospitalized mortality and early adverse outcomes**.** The restricted cubic splines were plotted by using 5 knots placed at 5th, 25th, 50th, 75th, 95th percentiles of FBG levels, and the cutoff of 7.0 mmol/L was set as the reference. The solid line shows the hazard ratios and the dash lines show the upper and lower 95% confidence interval (95% CI). The models adjusted for age, sex, chronic pulmonary disease, hypertension, diabetes mellitus, cardiovascular disease, chronic kidney disease, malignancy, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase, estimated glomerular filtration rate, hematocrit, Neutrophil-lymphocyte ratio, activated partial thromboplastin time, antibiotics treatment and corticosteroids treatment, except for the stratification variable itself.
Note: Noncolor in print version

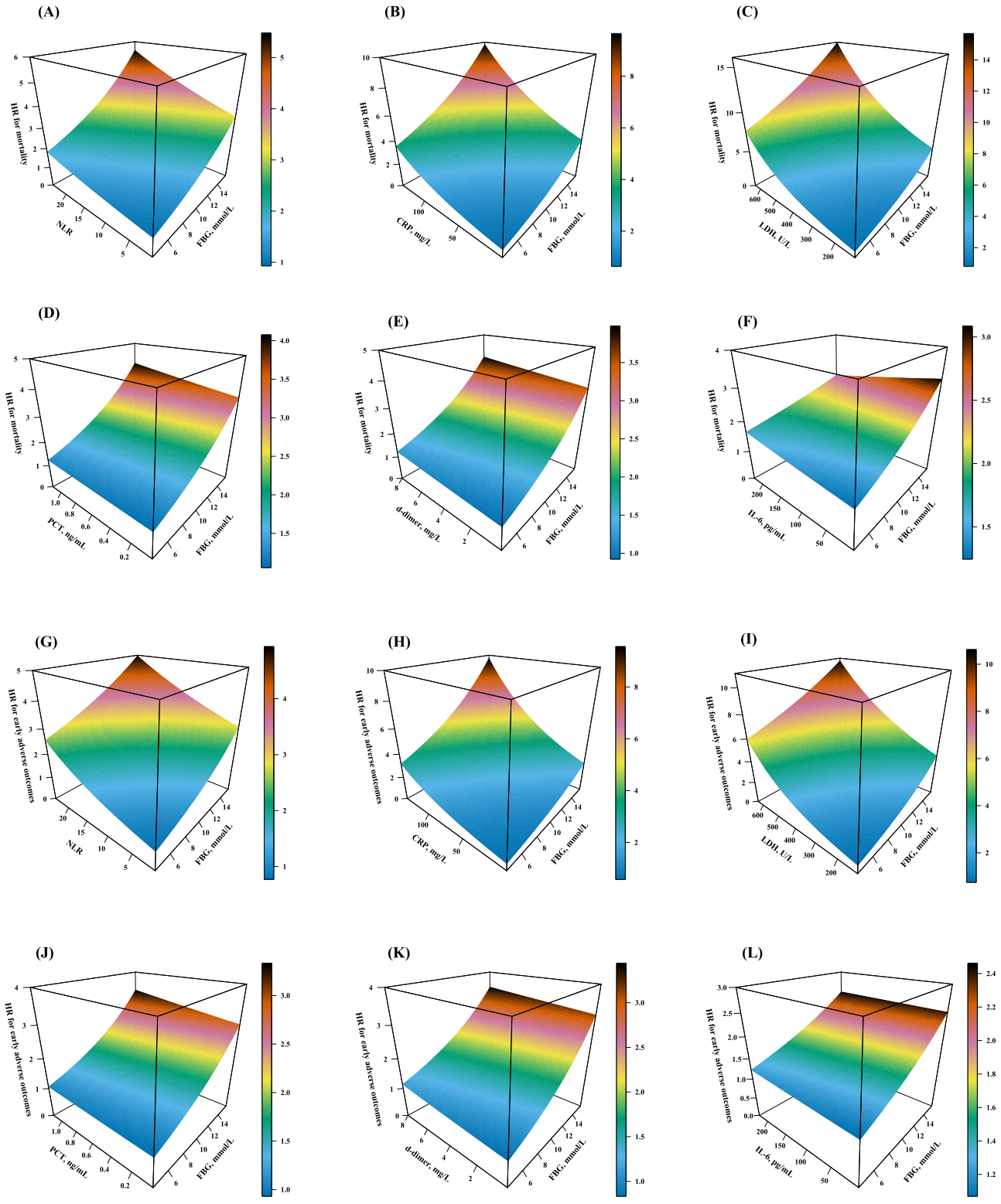


**Figure 2.** Three-dimensional joint effects trend plots of FBG with inflammation and coagulation markers and cytokines on risk for COVID-19 mortality and poor early outcomes. The models adjusted for age, sex, chronic pulmonary disease, hypertension, diabetes mellitus, cardiovascular disease, chronic kidney disease, malignancy, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase, estimated glomerular filtration rate, hematocrit, Neutrophil-lymphocyte ratio, activated partial thromboplastin time, antibiotics treatment and corticosteroids treatment. CRP, C-reactive protein; HR (95% confidence interval), hazard ratios ((95% CI); IL-6, interleukin-6; LDH lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin. Note: Noncolor in print version

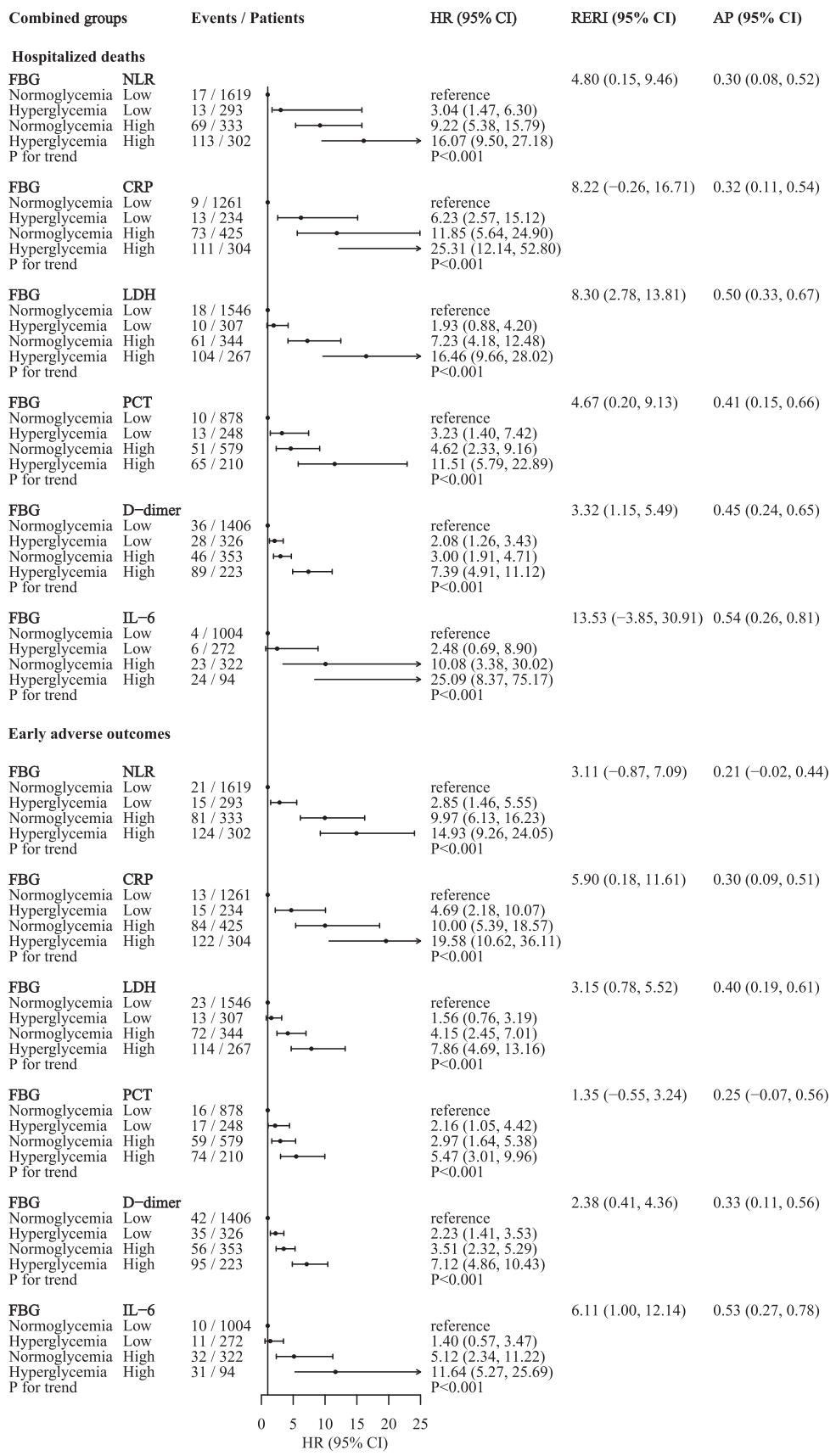


**Figure 3.** The quantitative additive interactions and joint effects of FBG with inflammation and coagulation markers and cytokines on risk for COVID-19 mortality and poor early outcomes. The models adjusted for age, sex, chronic pulmonary disease, hypertension, diabetes mellitus, cardiovascular disease, chronic kidney disease, malignancy, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase, estimated glomerular filtration rate, hematocrit, Neutrophil-lymphocyte ratio, activated partial thromboplastin time, antibiotics treatment and corticosteroids treatment. AP, the attributable proportion due to interaction; CRP, C-reactive protein; FBG, fasting blood glucose; HR (95% confidence interval), hazard ratios ((95% CI); IL-6, interleukin-6; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin; RERI, the relative excess risk due to interaction. Note: Noncolor in print version

**Table 1**
Basic characteristics across alive and died COVID-19 patients.

| Characteristics | N | Alive (N=2343) | Died (N=212) | *P* value |
|---|---|---|---|---|
| Age, (years) | 2555 | 59.0 (47.0, 67.0) | 69.0 (62.0, 78.0) | <0.0001 |
| Sex (Male, %) | 2555 | 1072 (45.8%) | 150 (70.8%) | <0.0001 |
| **Comorbidity** | | | | |
| Cardiovascular disease | 2555 | 319 (13.6%) | 59 (27.8%) | <0.0001 |
| Hypertension | 2555 | 989 (42.2%) | 107 (50.5%) | 0.020 |
| Diabetes mellitus | 2555 | 419 (17.9%) | 78 (36.8%) | <0.0001 |
| Chronic pulmonary disease | 2555 | 46 (2.0%) | 7 (3.3%) | 0.190 |
| Malignancy | 2555 | 101 (4.3%) | 20 (9.4%) | 0.001 |
| Chronic kidney disease | 2555 | 51 (2.2%) | 20 (9.4%) | <0.0001 |
| **Complete blood count** | | | | |
| Red blood cell, $\times 10^9$/L | 2547 | 4.1 (3.7, 4.4) | 4.1 (3.7, 4.5) | 0.535 |
| Hemoglobin, g/L | 2547 | 125.0 (115.0, 137.0) | 128.0 (117.0, 140.0) | 0.194 |
| Hematocrit, fl | 2547 | 0.37 (0.34, 0.40) | 0.38 (0.35, 0.41) | 0.292 |
| White blood cell, $\times 10^9$/L | 2547 | 5.5 (4.3-6.9) | 8.6 (6.0-11.5) | <0.0001 |
| Neutrophil count, $\times 10^9$/L | 2547 | 3.5 (2.6-4.7) | 7.3 (5.1-10.3) | <0.0001 |
| Lymphocyte count, $\times 10^9$/L | 2547 | 1.3 (0.9-1.7) | 0.6 (0.4-0.8) | <0.0001 |
| Blood platelets, $\times 10^9$/L | 2547 | 220.0 (171.0-278.0) | 161.0 (108.0-229.0) | <0.0001 |
| Neutrophil-lymphocyte ratio | 2547 | 2.57 (1.74-4.30) | 12.72 (7.78-22.00) | <0.0001 |
| **Coagulation indices** | | | | |
| aPTT, s | 2153 | 36.4 (33.6, 39.8) | 38.2 (34.0, 44.0) | 0.001 |
| Fibrinogen, g/L | 2153 | 3.9 (3.2, 4.8) | 4.4 (3.1, 5.3) | 0.720 |
| d-dimer, mg/L | 2315 | 0.4 (0.2, 1.1) | 4.3 (0.8, 8.0) | <0.0001 |
| **Inflammation indices** | | | | |
| C-reactive protein, mg/L | 2240 | 7.2 (3.1-33.1) | 84.8 (46.7-122.3) | <0.0001 |
| Lactate dehydrogenase, U/L | 2480 | 201.0 (167.0-266.0) | 498.0 (362.0-616.0) | <0.0001 |
| Procalcitonin, ng/ml | 1931 | 0.08 (0.05-0.13) | 0.34 (0.14-0.69) | <0.0001 |
| **Cytokines** | | | | |
| IL-4, pg/ml | 1375 | 2.4 (1.7, 3.5) | 2.1 (1.7, 2.8) | 0.795 |
| IL-6, pg/ml | 1692 | 5.8 (3.1, 16.6) | 70.1 (35.2, 615.0) | <0.0001 |
| IL-10, pg/ml | 1375 | 3.8 (2.9, 5.0) | 7.5 (4.9, 13.1) | <0.0001 |
| TNF-$\alpha$, pg/ml | 1375 | 2.8 (2.1, 4.0) | 2.4 (2.0, 2.9) | 0.249 |
| IFN-$\gamma$, pg/ml | 1375 | 2.4 (1.8, 3.4) | 2.2 (1.7, 2.9) | 0.879 |
| **Biochemical parameters** | | | | |
| Fasting blood glucose, mmol/L | 2555 | 5.6 (5.0, 6.6) | 7.8 (6.3, 11.0) | <0.0001 |
| Total cholesterol, mmol/L | 2355 | 4.2 (3.6, 4.9) | 3.7 (3.1, 4.3) | <0.0001 |
| Triglyceride, mmol/L | 2355 | 1.3 (1.0, 1.8) | 1.4 (1.1, 2.0) | 0.132 |
| HDL-C, mmol/L | 2355 | 1.1 (0.9, 1.3) | 0.8 (0.7, 1.0) | <0.0001 |
| LDL-C, mmol/L | 2355 | 2.4 (1.9, 2.9) | 2.1 (1.6, 2.6) | <0.0001 |
| ALT, U/L | 2555 | 28.0 (18.0, 47.0) | 34.0 (22.0, 55.0) | 0.0002 |
| AST, U/L | 2555 | 26.0 (20.0, 37.0) | 44.0 (31.0, 61.0) | <0.0001 |
| Creatinine, $\mu$mol/L | 2555 | 67.8 (57.0, 79.6) | 76.5 (63.4, 97.4) | <0.0001 |
| eGFR, ml/ min/l.73m$^2$ | 2555 | 95.0 (83.0, 105.0) | 83.0 (65.0, 96.0) | <0.0001 |
| **In-hospital treatment** | | | | |
| High-flow nasal cannula oxygen | 2555 | 73 (3.1%) | 68 (32.1%) | <0.0001 |
| Noninvasive ventilation | 2555 | 34 (1.5%) | 126 (59.4%) | <0.0001 |
| Anti-viral treatment | 2555 | 2019 (86.2%) | 190 (89.6%) | 0.160 |
| Antibiotics treatment | 2555 | 1596 (68.1%) | 194 (91.5%) | <0.0001 |
| Corticosteroids treatment | 2555 | 343 (14.6%) | 117 (55.2%) | <0.0001 |

Abbreviations: ALT: Alanine transaminase; AST: aspartate aminotransferase; aPTT: activated partial thromboplastin time; COVID-19: coronavirus disease 2019; eGFR: estimated glomerular filtration rate; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; IL: interleukin; TNF: tumor necrosis factor; IFN; interferon.
Notes: Continuous and category variables between alive and dead patients were compared by t-test or Mann-Whitney U test and chi-square test respectively according to the variable types and the distribution characteristics, and data were presented as median (the first and third IQR) and counts (%) respectively.

in women. In particular, we found strong joint effects of hyperglycemia, inflammation, coagulation, and cytokines on hospitalized mortality and poor early outcomes.

Previous studies usually treated glucose as dichotomous or categorical variables based on the clinical cutoff in the models and mainly focused on dichotomous hyperglycemia. Ciardullo et al. found diabetes was significantly associated with in-hospital mortality only if biomarkers of inflammation and procoagulation conditions at admission were taken into consideration (Ciardullo et al., 2021), whereas diabetes was not significantly associated with mortality when only considering demographic variables and pre-existing comorbidities. However, the associations were the strongest in our large study when only adjusting for age, sex, and all comorbidities, and the associations were attenuated but still robustly significant when further adjusting for other laboratory parameters, including inflammation indices of NLR. The results of additional sensitivity analysis by further adjusting for CRP, LDH, and d-dimer confirmed that the associations of hyperglycemia with prognosis were independent of inflammation and procoagulation again. This was in line with the results that the impact of hyperglycemia was independent of inflammation in patients with COVID-19 with diabetes (Vasbinder et al., 2022). Our findings indicated that FBG might be a better indicator than diabetes history. Vasbinder et al. (Vasbinder et al., 2022) also found the association between diabetes and outcomes was largely mediated by hyperinflammation as assessed by suPAR levels. Klonoff et al. (Klonoff et al., 2021) found individuals with hypoglycemia (FBG $<$ 3.9 mmol/L) were related to 2.2-fold increased risk for mortality in non-ICU inpatients. Zhu et al. also found that when compared with individuals in the second quintile (4.74 to 5.21

**Table 2**
Associations of FBG with hospitalized mortality and early adverse outcomes in patients with COVID-19

| | Events / Patients (%) | HR (95% CI) | |
|---|---|---|---|
| | | Model 1 | Model 2 |
| **Hospitalized deaths** | | | |
| FBG, mmol/L | | | |
| ≤4.0 | 4 / 28 (14.3%) | 4.23 (1.51, 11.85) | 3.13 (1.07, 9.19) |
| 4.0-6.1 | 43 / 1594 (2.7%) | 1.00 (Ref.) | 1.00 (Ref.) |
| 6.1-7.8 | 60 / 502 (12.0%) | 3.95 (2.66, 5.88) | 2.52 (1.64, 3.89) |
| 7.8-10.0 | 41 / 215 (19.1%) | 6.18 (3.96, 9.63) | 2.59 (1.57, 4.27) |
| >10.0 | 64 / 216 (29.6%) | 12.92 (8.17, 20.45) | 4.44 (2.59, 7.62) |
| *P* for quadratic trend | | <0.0001 | 0.01 |
| **Early adverse outcomes** | | | |
| FBG, mmol/L | | | |
| ≤4.0 | 4 / 28 (14.3%) | 3.27 (1.17, 9.12) | 2.09 (0.71, 6.72) |
| 4.0-6.1 | 53 / 1594 (3.3%) | 1.00 (Ref.) | 1.00 (Ref.) |
| 6.1-7.8 | 69 / 502 (13.8%) | 3.39 (2.35, 4.88) | 2.32 (1.57, 3.45) |
| 7.8-10.0 | 45 / 215 (20.9%) | 5.03 (3.32, 7.61) | 2.05 (1.28, 3.30) |
| >10.0 | 70 / 216 (32.4%) | 9.99 (6.49, 15.38) | 3.72 (2.25, 6.15) |
| *P* for quadratic trend | | <0.0001 | 0.059 |

Abbreviations: aPTT: activated partial thromboplastin time; ALT: alanine transaminase; AST: aspartate aminotransferase; COVID-19: coronavirus disease 2019; eGFR: estimated glomerular filtration rate; FBG: fasting blood glucose; NLR: Neutrophil-lymphocyte ratio.
Notes: Model 1 adjusted for age, sex, and baseline comorbidities (including chronic pulmonary disease, malignancy, cardiovascular disease, hypertension, diabetes mellitus, and chronic kidney disease). Model 2: adjusted model 1 plus total cholesterol, triglyceride, ALT, AST, eGFR, hematocrit, NLR, aPTT, antibiotics treatment and corticosteroids treatment.

mmol/L), those in the first quintile (FBG < 4.74 mmol/L) and the third to highest quintiles (≥5.21 mmol/L) had a higher risk of severe and critical condition in 293 patients with no diabetes mellitus and with COVID-19, which showed a J-shaped association (Zhu et al., 2020). Our results supported and extended previous findings. We further used restricted cubic splines nested in Cox regression to fit the J-shaped dose-response curve, and observed that the risk of hospitalized mortality and poor early outcomes linearly increased when FBG levels exceeded the nadir of 5 mmol/L. Besides, low FBG was also associated with a high dose-response risk for hospitalized death and poor early outcomes. Although the mechanisms were not fully understood, it was possible that low levels of glucose might result in insufficient energy for fighting against the virus at cellular levels, induce enhanced oxidative and impaired immune responses, and finally cause a series of bad health conditions (Li et al., 2020; Zhu et al., 2020). Another alternative interpretation was that hypoglycemia might occur in patients with COVID-19 with sepsis. However, it is well known that in condition of sepsis, hypoglycemia is a powerful predictor of poor prognosis (Mitsuyama et al., 2022; Wang et al., 2021). Therefore, regardless of its cause, hypoglycemia was an independent risk factor for poor prognosis in patients with COVID-19. Correction of hypoglycemia may improve outcomes.

In particular, there were no obvious threshold effects for hypoglycemia and hyperglycemia. However, one study in 417 Kuwaiti patients (aged 45.4 ± 17.1 years) with COVID-19 reported a nonlinear trend that the dose-response curve slope of risk for being admitted to ICU became very flat when FBG exceeded 10 mmol/L (Alahmad et al., 2020). Small sample size, single outcome definition of ICU, insufficient outcome events, and younger patients in the Kuwait study could have contributed to inconsistent results. In fact, the exposure-response figure in that study showed that there were few patients whose FBG were beyond 10 mmol/L or below 4 mmol/L, and the odds ratios of ICU admission might be underestimated owing to insufficient statistical power. For example, in the dichotomous and categorical models, those with FBG ≥ 7 mmol/L had 15-fold and 19-fold risks of ICU admission, whereas the upper limits of 95% confidence intervals were up to 32-fold and 51-fold risk. In contrast, our results might fully reveal the dose-response relationships; that is, too low or too high FBG at admission was associated with increased risk of mortality and poor early outcomes. However, considering the retrospective nature, there was a possibility that hyperglycemia may have no causal effect on the outcomes, but may simply represent a parallel and proportional manifestation of more serious clinical conditions at admission.

Notably, the stratification analyses found a strong multiplicative interaction of FBG with sex. The dose-response associations were more evident in women, especially when FBG was higher than 5 mmol/L. To our knowledge, this study found the modification effect of sex on the above dose-response relationship for the first time. However, we could not directly compare our results with previous studies because no similar findings were reported. Nevertheless, many studies found that diabetes mellitus and hyperglycemia conferred a greater risk for many diseases, such as cardiovascular diseases, in women than in men (Ahn et al., 2018; Barrett-Connor et al., 1991; Xu et al., 2019), which partly supported our results. Although the underlying mechanisms were unclear, it was possible that the relatively lower mortality for women without hyperglycemia than that of men contributed to the sex difference (Barrett-Connor et al., 1991). For instance, in the present study, the mortality was 1.85% for women without hyperglycemia, whereas it reached up to 7.53% for men without hyperglycemia. However, it seemed that there was no significant difference between women with hyperglycemia (18.07%) and men with hyperglycemia (24.28%).

The most interesting finding of the current study was that it was the first time, to our knowledge, that strong joint effects of hyperglycemia were found with NLR, CRP, LDH, d-dimer, and IL-6, which indicated that hyperglycemia might play a synergistic role in the process of inflammation, immune response, and cytokine storm among COVID-19 patients. This wide range of joint effects means that we might have seriously underestimated the adverse effects of hyperglycemia on risk for COVID-19 mortality and poor early outcomes. Many previous studies supported the findings of the abovementioned strong additive interactions and joint effects(Chen et al., 2020; Dhar et al., 2021; Lim et al., 2021;

Zhao et al., 2020; Zheng et al., 2021). Three early studies suggested that immune response and cytokine profiles were dramatically increased in patients with diabetes mellitus (Lim et al., 2021; Zhao et al., 2020; Zheng et al., 2021). Two other studies indicated that inflammation and hypercoagulation were predominant risk factors of severity and mortality in patients with COVID-19 with diabetes mellitus (Chen et al., 2020; Dhar et al., 2021). In fact, we found that the independent effect of hyperglycemia might be relatively small. A more important role of hyperglycemia might be the potential impact of amplification on inflammation, hyperimmune response, and cytokines. Our results showed that a small increase in FBG was associated with increased mortality. This effect might be due to glucose variation itself or a possible interaction of dysglycemia with other markers. An alternative interpretation was the increased inflammation may cause hyperglycemia through increased counter-regulatory hormone secretion.

This is the first large retrospective study to demonstrate a sex-specific J-shaped association of FBG with mortality and poor early outcomes in patients with COVID-19, regardless of pre-existing diabetes mellitus. Furthermore, we believe we are among the first to find the strong joint effects of hyperglycemia with inflammation, coagulation, and cytokines. However, our study has several limitations. First, considering the retrospective design of this study, the causal inference should be cautious. Second, there were limited cases and patients in hypoglycemia groups, even though the overall cases and sample size were relatively large. However, we further confirmed the robust J-shaped associations by fitting strict cubic splines, which might greatly enhance the results. Third, in almost all the relevant studies, we investigated a single spot FBG on the morning of the second day after admission, rather than mean FBG control levels like glycosylated hemoglobin A1c (HbA1c) or continuous monitoring of FBG levels. The FBG values on the morning of the second day might be affected by treatment (e.g., corticosteroids) as well as by disease progression itself, but they were the earliest available values because random plasma glucose on the day of admission was usually not tested among the patients. Besides, the absence of HbA1c data did not allow one to differentiate between undiagnosed diabetes and stress hyperglycemia. Several studies showed that patients with stress hyperglycemia have worse in-hospital mortality related to different causes than did known or undiagnosed diabetic patients (Capes et al., 2000; Mi et al., 2022). However, a large study has indicated that FBG at admission was highly correlated with mean glucose levels (Klonoff et al., 2021). Meanwhile, a wealth of studies indicated that FBG levels at admission might represent the current magnitude of hyperglycemia at the time and could better predict prognosis (Carrasco-Sánchez et al., 2021; Chen et al., 2021; Coppelli et al., 2020; Huang et al., 2020; Klonoff et al., 2021; Lazarus et al., 2021; Wang et al., 2020; Yang et al., 2021). In addition, our conclusion was limited by some unadjusted confounders, including absence of HbA1c, body mass index, and lifestyles, and massive missing data such as IL-4, IL-10, and TNF-$\alpha$, as well as potential residual bias, all of which might lead to overestimation for hazard risk.

In conclusion, our study demonstrated J-shaped associations of admission FBG with risk of hospitalized mortality and poor early composite outcomes, with a nadir at 5 mmol/L of FBG. In particular, the J-shaped dose-response associations were more evident in women when FBG exceeded 5 mmol/L. Most importantly, we found strong joint effects of hyperglycemia, inflammation, coagulation, and cytokines, which might provide new insights into the role of FBG in hospitalized mortality and poor early outcomes in patients with COVID-19. However, large prospective randomized studies are warranted to confirm our results.

## Declarations of competing interest

The authors declare that none of competing financial interests or personal relationships could have appeared to influence the work reported in this paper. The authors declare that they have no conflicts of interest.

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## Ethical Approval

This study was approved by the ethical committee of Wuhan Red Cross hospital and Wuhan Union Hospital, Tongji medical college, Huazhong University of Science and Technology. Written informed consent was waived by the Ethics Committee due to emerging infectious disease and the retrospective and anonymous nature of the study.

## Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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## Author contributions

X. Lai, S. Deng, X. Zhang and K. Huang contributed to the concept of the study and design. S. Deng. L. Hu, R. Chen, M. Chen, M. Liang collected the data and confirmed the accuracy. X. Lai, S. Deng, J. Hou, X. Zhang and K. Huang conducted the statistical analysis. X. Lai was in charge of the manuscript draft. X. Lai, S. Deng, X. Zhang and K. Huang contributed the critical reversion of the manuscript. K. Huang, X. Zhang supervised the study. All authors reviewed and approved the final manuscript.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.05.060.

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