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PROGNOSTIC VALUE OF FERROPTOSIS-RELATED BIOMARKERS ACSL4 AND GPX4 IN CRITICALLY ILL PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

PROGNOSTIČKA VREDNOST BIOMARKERA ACSL4 I GPX4 POVEZANIH SA FEROPTOZOM KOD KRITIČNO BOLESNIH PACIJENATA: SISTEMATSKI PREGLED I META-ANALIZA

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Summary

Background: Ferroptosis is a regulated form of cell death... Although ferroptosis has been increasingly implicated in influenza A virus (IAV)-induced lung injury, direct clinical evidence in influenza A patients remains scarce. This systematic review and meta-analysis aimed to evaluate the prognostic significance of serum ACSL4 and GPX4 levels in critically ill patients with severe infections, and to assess the potential generalizability of these findings to influenza A-related critical illness.

Methods: A systematic search of PubMed, Embase, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang Data, and China Biology Medicine Disc (CBM) was conducted from database inception to March 2026. Observational studies evaluating the association between serum ACSL4 and/or GPX4 levels and clinical outcomes in critically ill patients were included. Study quality was assessed using the Newcastle–Ottawa Scale (NOS). Meta-analysis was performed using RevMan 5.4 and Stata 16.0. Odds ratios (ORs) and 95% confidence intervals (CIs) were pooled using random-effects models. Diagnostic performance was evaluated using receiver operating characteristic (ROC) data where available. Heterogeneity, sensitivity analyses, and publication bias assessments were conducted according to standard methodological approaches.

Results: A total of 42 records were identified, and two high-quality prospective cohort studies involving 437 critically ill patients were included. NOS scores ranged from 8 to 9 points. Despite the limited number of included studies, meta-analysis demonstrated that ferroptosis-re-

Kratak sadržaj

Uvod: Feroptoza je regulisani oblik ćelijske smrti... Iako je feroptoza sve više povezana sa oštećenjem pluća izazvanim virusom gripa A (IAV), direktni klinički dokazi kod pacijenata obolelih od gripa A ostaju oskudni. Ovaj sistematski pregled i meta-analiza imali su za cilj da procene prognostički značaj nivoa serumskog ACSL4 i GPX4 kod kritično bolesnih pacijenata sa teškim infekcijama i da procene potencijalnu generalizaciju ovih nalaza na kritične bolesti povezane sa gripom A.

Metode: Sistematska pretraga baza podataka PubMed, Embase, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang Data i China Biology Medicine Disc (CBM) sprovedena je od nastanka baze podataka do marta 2026. godine. Uključene su opservacione studije koje procenjuju povezanost izme u nivoa ACSL4 i/ili GPX4 u serumu i kliničkih ishoda kod kritično bolesnih pacijenata. Kvalitet studije procenjen je korišćenjem Njukasl-Otavske skale (NOS). Meta-analiza je sprovedena korišćenjem RevMan 5.4 i Stata 16.0. Odnosi šansi (OR) i 95% intervali poverenja (CI) su objedinjeni korišćenjem modela slučajnih efekata. Dijagnostičke performanse su procenjene korišćenjem podataka ROC (receiver Operating Characteristic) gde su bili dostupni. Analize heterogenosti, osetljivosti i procene pristrasnosti objavljivanja sprovedene su u skladu sa standardnim metodološkim pristupima.

Rezultati: Ukupno je identifikovano 42 zapisa, a uključene su dve visokokvalitetne prospektivne kohortne studije koje su obuhvatile 437 kritično bolesnih pacijenata. NOS rezultati su se kretali od 8 do 9 poena. Uprkos ograničenom broju uključenih studija, meta-analiza je pokazala

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lated biomarkers were significantly associated with short-term mortality. Reduced serum GPX4 (OR=1.076, 95% CI: 1.036–1.119), reduced ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (OR=1.258, 95% CI: 1.161–1.364), and elevated serum ACSL4 (OR=4.00, 95% CI: 2.00–8.00) were independently associated with adverse outcomes. The pooled OR was 1.76 (95% CI: 1.03–3.01), indicating a significantly increased risk of short-term mortality in patients exhibiting ferroptosis pathway dysregulation. Regarding prognostic performance, ACSL4 alone yielded an area under the curve (AUC) of 0.713, whereas GPX4 alone showed limited predictive ability (AUC=0.573). Combined assessment of GPX4 and GSH/GSSG improved predictive performance substantially (AUC=0.841). Sensitivity analyses confirmed the robustness of the findings.

Conclusion: Dysregulation of ferroptosis-related biomarkers—specifically, elevated ACSL4 and reduced GPX4 is associated with adverse short-term outcomes in critically ill patients with severe infections. Combined evaluation of ferroptosis-related markers may improve risk stratification and prognostic assessment. These findings support the potential clinical utility of the ACSL4/GPX4 axis as a biomarker system and highlight ferroptosis as a promising therapeutic target in critical illness.

Keywords: ACSL4, GPX4, ferroptosis, critical illness, sepsis, community-acquired pneumonia, prognosis

da su biomarkeri povezani sa feroptozom značajno povezani sa kratkoročnim mortalitetom. Smanjen serumski GPX4 (OR=1,076, 95% CI: 1,036–1,119), smanjen odnos redukovanog glutationa (GSH) i oksidovanog glutationa (GSSG) (OR=1,258, 95% CI: 1,161–1,364) i povišen serumski ACSL4 (OR=4,00, 95% CI: 2,00–8,00) bili su nezavisno povezani sa nepovoljnim ishodima. Zbirni OR je bio 1,76 (95% CI: 1,03–3,01), što ukazuje na značajno povećan rizik od kratkoročnog mortaliteta kod pacijenata sa disregulacijom feroptoznog puta. što se tiče prognostičkih performansi, ACSL4 sam po sebi je dao površinu ispod krive (AUC) od 0,713, dok je GPX4 sam po sebi pokazao ograničenu prediktivnu sposobnost (AUC=0,573). Kombinovana procena GPX4 i GSH/GSSG značajno je poboljšala prediktivne performanse (AUC=0,841). Analize osetljivosti potvrdile su robusnost nalaza.

Zaključak: Disregulacija biomarkera povezanih sa feroptozom – tačnije, povišen ACSL4 i smanjen GPX4 – povezana je sa nepovoljnim kratkoročnim ishodima kod kritično obolelih pacijenata sa teškim infekcijama. Kombinovana evaluacija markera povezanih sa feroptozom može poboljšati stratifikaciju rizika i prognostičku procenu. Ovi nalazi podržavaju potencijalnu kliničku korisnost ACSL4/GPX4 ose kao sistema biomarkera i ističu feroptozu kao obeć avajuću terapijsku metu kod kritičnih bolesti.

Ključne reči: ACSL4, GPX4, feroptoza, kritična bolest, sepsa, vanbolnički stečena pneumonija, prognoza

Introduction

Influenza A is a major global respiratory infectious disease. Some patients can rapidly progress to severe or critical illness, manifesting as acute respiratory distress syndrome (ARDS), multiple organ failure, or death (1). Early identification of patients at high risk of severe disease is crucial for optimizing clinical decisions and reducing mortality. In recent years, the value of traditional inflammatory markers such as C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6) in predicting the severity of respiratory tract infection has been widely discussed. However, these markers lack pathogen specificity and are difficult to reflect the deep pathophysiological mechanism of disease progression. Li et al. (2) analyzed the metabolomics of 339 patients with influenza A H1N1 and found that the progressive disorders of glycerophospholipid metabolism and taurine and hypotaurine metabolism pathways were closely related to critical illness transformation, suggesting that abnormal lipid metabolism may be an important internal clue for severe influenza. However, these metabolites are limited by equipment conditions and analytical throughput, which is difficult to promote in primary clinical practice. There is an urgent need to find blood biomarkers with higher accessibility and more direct correlation with pathological mechanisms.

Ferroptosis is A regulatory cell death mode driven by iron-dependent lipid peroxidation, and its core regulatory mechanism involves two key nodes, acyl-coa synthetase long-chain family member 4 (ACSL4) and glutathione peroxidase 4 (GPX4) (3). ACSL4 catalyzes the esterification of polyunsaturated fatty acids (PUFA) to phosphatidylethanolamine, which provides a substrate for lipid peroxidation and is the promoter of ferroptosis. GPX4 relies on glutathione (GSH) to reduce lipid hydroperoxides to lipid alcohols, and is the core inhibitor of ferroptosis (1). In the field of viral infection, there is increasing evidence that ferroptosis is involved in the pathological process of lung injury caused by influenza A virus (IAV). Ouyang et al. (4) demonstrated for the first time that IAV hemagglutinin (HA) protein drives ferritin autophagy to promote ferroptosis by interacting with autophagy receptors NCOA4 and TAX1BP1, thus facilitating virus replication. GPX4 and ferritin heavy chain (FTH1) protein expression decreased significantly after infection. In response, Guo et al. (5) revealed the mechanism of RAGE-ACSL4/POR/GPX4 axis driving ferroptosis and oxidative stress-induced lung injury in viral infection, and found that RAGE activation could up-regulate ACSL4 expression and down-regulate GPX4 expression, while RAGE inhibitor FPS-ZM1 could reverse this effect. The review by Letafati et al. (6) further integrated the results of multiple studies and pointed out that IAV infection not only weakened the host antioxidant defense through the inhibition of the NRF2-KEAP1 pathway, but also induced

mitochondrial fragmentation through PB2 and NS1 proteins, leading to the massive generation of reactive oxygen species (ROS) and the up-regulation of ACSL4 mRNA and protein expression. Mao et al. (7), in their systematic review of the role of ferroptosis in viral infections, suggested that the expression patterns of ferroptosis markers in different viral diseases are highly heterogeneous: In IAV infection, ACSL4 up-regulation and GPX4 down-regulation are typical features, while in some RNA virus infections, damage to the SLC7A11/GPX4 axis is the dominant pathway (8). This difference suggests that the ferroptosis regulatory network is pathogen specific.

From the perspective of clinical transformation, ACSL4 and GPX4 are the key molecules in the ferroptosis pathway, and the correlation between their serum levels and the severity of the disease has been verified in some clinical studies. Zhu et al. (9) demonstrated in a prospective cohort study of 267 patients with severe community-acquired pneumonia (CAP) that decreased serum GPX4 levels were significantly associated with increased 30-day mortality, and GPX4 alone had an AUC of 0.778 (95% CI: When combined with GSH/GSSG ratio, the AUC increased to 0.841 (95%CI: 0.778-0.903), and the multivariate Logistic regression OR value of low GPX4 group was 0.929 ($P < 0.001$), which was an independent risk factor for death. However, this study focused on the overall CAP population, did not stratianalyze by pathogen, and did not include ACSL4 testing. Jankauskas et al. (8) found that COVID-19 could induce ferroptosis in human endothelial cells accompanied by oxidative stress, further supporting the widespread existence of ferroptosis in viral respiratory diseases.

Although the pathological mechanism of ferroptosis markers in influenza A-induced lung injury has been established in preclinical models, direct clinical evidence from influenza A cohorts remains insufficient. Given the shared ferroptosis pathophysiology across severe respiratory infections, we conducted this systematic review and meta-analysis to (i) quantitatively evaluate the prognostic value of serum ACSL4 and GPX4 in critically ill patients with severe infections (primarily CAP and sepsis), and (ii) assess the potential applicability of these findings to influenza A-related critical illness through pathophysiological inference. We explicitly acknowledge that the included studies do not comprise influenza A-specific populations, and thus the conclusions regarding influenza A represent mechanistically grounded extrapolation rather than direct evidence.

Materials and Methods

Study Design

This study adopted the methodological framework of systematic review and meta-analysis. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) reporting standards (10) were strictly followed for design and writing. in view of the fact that all the included Studies were observational clinical studies, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) (11) was also referred to in this study. To ensure the completeness and transparency of methodological reporting.

Literature search strategy

Search the database

A combination of computer retrieval and manual retrieval was used to search the following 7 Chinese and English electronic databases from the establishment of the database to March 31, 2026: (1) PubMed; (2) Embase; (3) Web of Science core collection; (4) Cochrane Library; (5) China National Knowledge Infrastructure (CNKI); (6) Wanfang Data Knowledge Service Platform; (7) Chinese Biomedical Literature Database (CBM). In addition, the reference lists of the included studies were searched manually, and the relevant clinical trials that have been registered but not yet published were searched on the international clinical trial registration platform (ClinicalTrials.gov and the Chinese Clinical trial registry ChiCTR) to reduce the missing literature as much as possible.

Construction of search terms

Search strategies were developed using a combination of MeSH terms and keywords, following the PICOS framework. English search terms include: influenza A OR influenza A virus OR H1N1 OR H3N2 OR IAV) AND (ACSL4 OR acyl-CoA synthetase long-chain family member 4) AND (GPX4 OR glutathione peroxidase 4) AND (severity OR severe OR critical OR prognosis OR prediction OR outcome OR mortality OR ICU OR ARDS. MeSH and Emtree were used for PubMed and Embase searches, respectively. The search strategy was reviewed and optimized by medical information experts.

Literature screening

Literature screening followed the independent double principle. Two researchers independently screened the literature according to the predefined inclusion and exclusion criteria. Firstly, the literature

that obviously did not meet the requirements was excluded by reading the title and abstract, and then the full text of the literature that may meet the requirements was obtained for further review. Any discrepancies that arose during screening were resolved by discussion between the two investigators, and if agreement was not reached, arbitration was performed by the third senior investigator. EndNote X9 literature management software was used for literature deduplication and screening process management. The literature screening process is presented in the form of a PRISMA 2020 flowchart.

Inclusion and exclusion criteria

The PICOS framework was used to formulate the inclusion criteria:

P (study population): Hospitalized patients with laboratory-confirmed influenza A virus (including H1N1, H3N2 and other subtypes) infection, regardless of age and underlying disease status.

I (index detection): peripheral blood samples were collected after admission or early after diagnosis (within 7 days) to detect serum ACSL4 and/or GPX4 levels, and the detection methods included but were not limited to enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence immunoassay (ECLIA), immunoturbidimetry, etc.

C (control comparison): divided into severe group and non-severe group according to the severity of influenza A. The severe group was defined according to the latest edition of »Influenza Diagnosis and Treatment Protocol« issued by Health Commission of China and relevant international guidelines, meeting at least one of the following criteria: (1) admission to the intensive care unit (ICU); (2) requiring mechanical ventilation or extracorporeal membrane oxygenation (ECMO) for respiratory support; (3) complicated with ARDS; (4) septic shock; (5) Death. The non-severe group was defined as patients admitted to general wards who did not meet any of the above severe criteria.

O (outcome measure): the primary outcome measure was the difference in serum ACSL4 and GPX4 levels between the severe group and the non-severe group (measured as standardized mean difference SMD); The secondary outcome measures were the diagnostic efficacy parameters of ACSL4 and/or GPX4 in predicting the severity of influenza A, including sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and area under the summary receiver operating characteristic curve (AUC).

S (Study design): Observational clinical studies on the association of serum ACSL4 and/or GPX4 levels with disease severity in patients with influenza A, including cross-sectional studies, case-control studies, and cohort studies (prospective or retrospective), with or without reporting full 2×2 contingency table data on diagnostic test accuracy.

Exclusion criteria: (1) The research objects were animal experiments or in vitro cell experiments; (2) The subjects of the study were patients with acute lung injury caused by other clear causes (such as bacterial pneumonia, fungal pneumonia, tuberculosis, non-infectious ARDS, etc.), and no independent stratified analysis was conducted on patients with influenza A. (3) Duplicate publications (only the version with the most complete information or the largest sample size was retained); (4) studies with incomplete data, unable to extract effective data, and still unable to obtain after contacting the original authors; (5) conference abstracts, case reports, reviews, comments, dissertations and other non-original clinical research documents; (6) literature in non-Chinese and English languages.

Data extraction

Two investigators independently extracted data from the included studies using a pre-designed and pre-tested data extraction form, and cross-checked the data after completion of extraction. The main contents of the data extraction table included: (1) basic information: first author, publication year, country or region, study design type; (2) Characteristics of study population: sample size (total sample size and each subgroup sample size of severe or non-severe group), average age or age range, gender ratio, distribution of influenza A subtypes, and underlying diseases; (3) Detection indicators and methods: detection time point, specimen type, detection method and detection unit of ACSL4 and GPX4; (4) the definition of severe cases and the version of specific diagnostic guidelines referred to; (5) Outcome data: the mean ± standard deviation (M±SD) or median and interquartile range (MŠIQRĆ) of ACSL4 and GPX4 in severe group and non-severe group, 2×2 contingency table data; (6) Adjustment of confounding factors: whether multivariate Logistic regression analysis was performed and the adjusted OR value and 95%CI were reported. In the process of data extraction, if the original literature only reported the median and interquartile range, it was converted to mean and standard deviation. Disagreements during extraction were resolved by discussion or by adjudication by a third investigator.

Risk of bias assessment

Two researchers independently evaluated the risk of bias of the included studies using the

Newcastle-Ottawa scale (NOS). The NOS scale has 8 items for cohort studies and case-control studies, covering three dimensions of quality assessment: (1) selection of study population, including representative of exposure group, selection of control, identification of exposure, etc. The maximum score is 4; (2) comparability between groups, to evaluate whether the most important confounding factors were controlled in the design and analysis, with a maximum score of 2; (3) Outcome measures, including objectivity of outcome assessment and adequacy of follow-up time, can be scored up to 3 points. The total score of the NOS ranged from 0 to 9, with a total score ≥7 as low risk of bias (high quality), 4-6 as moderate risk of bias (moderate quality), and ≤3 as high risk of bias (low quality). For cross-sectional studies, the Cross-sectional Studies adaptation version of the NOS Scale was used for evaluation. The results of risk of bias assessment were presented in table form and cross-checked by two researchers independently. If there were any differences, they were resolved by discussion or consultation with a third researcher.

Literature quality evaluation

The criteria recommended by AHP were used to grade the overall evidence quality of the included studies, and at the same time, the clinical relevance and the evidence strength of each outcome index were combined to make a comprehensive judgment. The weight of evidence for each outcome indicator was divided into four levels: high, medium, low, and very low. The dimensions evaluated were risk of bias (based on NOS score), inconsistency (I^2 statistic and clinical heterogeneity), indirectness (how well participants, interventions, and outcome measures fit the target question), imprecision (width of confidence intervals and sample size), and publication bias (funnel plot symmetry). When there is significant heterogeneity with $I^2 > 50\%$, after exploring the source of heterogeneity, if the reasons for heterogeneity can be reasonably explained, the quality of evidence will not be degraded. Otherwise, it is degraded. The results of the evidence quality evaluation were presented in the form of a result summary sheet.

Statistical analysis

Meta-analysis was performed using RevMan 5.4 software (Cochrane Collaboration, London, UK) and Stata 17.0 software (StataCorp, College Station, TX, USA). For the comparison of serum ACSL4 and GPX4 levels between the severe group and the non-severe group, standardized mean difference (SMD) was used as the combined effect size index to eliminate the influence of inconsistent detection methods and measurement units among different studies, and 95% confidence intervals (CI) were reported. Cochran's Q test was used to evaluate the statistical heterogeneity among the included studies, and the test level was set at $\alpha = 0.10$. The I^2 statistic was used to quantitatively evaluate the degree of heterogeneity. When $I^2 \leq 50\%$ and $P \geq 0.10$, heterogeneity was considered acceptable, and the fixed effect model was used to merge the effect size. When $I^2 > 50\%$ or $P < 0.10$, significant heterogeneity was considered and effect sizes were pooled using a random effects model.

For studies reporting complete 2×2 contingency table data (sensitivity and specificity), bivariate model analysis was performed using midas command package in Stata 17.0 software. Considering the potential negative correlation between sensitivity and specificity, the model was fitted with linear mixed effects of sensitivity and specificity respectively, and the parameter was estimated after logit transformation. Then the pooled sensitivity, pooled specificity and their 95%CI were calculated, and the pooled receiver operating characteristic curve was drawn, and the pooled AUC and its 95%CI were calculated. The area under the SROC curve ranged from 0.5 to 0.7 indicating low predictive efficiency, from 0.7 to 0.9 indicating medium predictive efficiency, and ≥0.9 indicating high predictive efficiency. When significant heterogeneity ($I^2 > 50\%$) was present, prespecified subgroup analyses were performed to explore the source of heterogeneity. Subgroup analysis variables included: (1) influenza A subtype (H1N1 vs H3N2 vs unclassified); (2) study design (prospective cohort vs retrospective cohort vs cross-sectional study); (3) detection methods (ELISA vs ECLIA vs others); (4) Sample size (≥100 vs <100); (5) geographic region (Asia vs Europe vs North America); (6) Age stratification (adult vs child vs mixed). At the same time, meta-regression analysis based on LeClert et al. method was used to further explore the contribution of continuous covariates to heterogeneity. Sensitivity analysis was performed using the one-by-one exclusion method, that is, one study was excluded at a time and the effect size was combined again to observe the stability and directional change of the combined results. The fixed effect model and random effect model were used for combined analysis to compare the differences of results under the two models. Results were considered to be robust if the direction and significance of the pooled effect size remained consistent before and after the sensitivity analysis. Only high-quality studies with NOS score ≥7 (low risk of bias) were included for sensitivity analysis to assess the impact of risk of bias on the pooled results. For continuous outcome indicators (ACSL4 and GPX4 levels), Egger linear regression test was used to quantitatively assess funnel plot

asymmetry. For pooled analyses of diagnostic test accuracy, publication bias was assessed with the use of funnel plot asymmetry tests (weighted linear regression with the log variance of effect size as the independent variable and the log ratio of effect size as the dependent variable). The significance level for all the above tests was set at $P < 0.10$. If significant publication bias was detected, the snip-and-fill method was used to estimate the adjusted combined effect size. The significance level for all statistical analyses was set at a two-sided $P < 0.05$, except for special Settings for tests of heterogeneity and publication bias.

Results

Literature screening process

This systematic review followed the PRISMA 2020 statement for literature search and screening. PubMed, Embase, Cochrane Library, and Web of Science core Collection were systematically searched from the establishment of the database to April 13, 2026, and ClinicalTrials.gov clinical trial registration platform was also searched. A total of 42 related literatures were obtained by combining subject words and free words, and 28 literatures were left after removing the duplication by EndNote 21 software. Two researchers independently screened titles and abstracts, excluded 24 obviously irrelevant articles, and obtained 4 full texts for detailed evaluation. In the full-text screening stage, two studies were excluded according to the inclusion and exclusion criteria: one was animal experiment or in vitro cell research, and the other was non-influenza A population (mixed pathogen cohort, no independent stratified analysis of influenza A patients). Two studies that met the criteria were finally included (9, 12) (*Figure 1*).

Basic characteristics of the included studies

A total of 2 prospective observational studies were included in this study. The first study published by Zhu et al. (9) from China was a prospective observational cohort study, including 267 patients with severe community-acquired pneumonia (CAP) admitted to ICU, and the study period was from 2021 to 2023. All patients were diagnosed according to the American Thoracic Society (ATS) guidelines and judged as severe by pneumonia Severity Index (PSI) stage IV-V. Patients received standardized evidence-based treatment, including empirical broad-spectrum antibiotics, glucocorticoids, and supportive care (electrolyte and acid-base management, oxygen therapy, hemodynamic stabilization, mechanical ventilation, etc.). Patients with COVID-19 were additionally treated with antiviral drugs such as remdesivir. The patients were divided into survival group (188 cases, 70.4%) and death group (79 cases, 29.6%) according to 30-day prognosis. Serum GPX4 and GSH/GSSG ratio levels were measured within 24 hours after admission. The second study from China, published by Zeng et al (12) in 2025, was a single-center prospective observational study, including 170 septic patients, 49 non-septic ICU patients and 50 healthy subjects admitted to ICU from January to December 2023. Patients with Sepsis were diagnosed according to Sepsis-3 guidelines, including 69 patients with septic shock. All patients received routine ICU diagnosis and treatment, including blood routine, blood lactic acid, glomerular filtration rate, infection markers (hs-CRP, PCT) and bacterial culture. The levels of serum ferroptosis-related proteins (ACSL4, GPX4, PTGS2) and inflammatory factors (IL-1 , IL-6, IL-8, IL-10, TNF-α, CXCL2, MCP-1, CL-11) were detected to evaluate their value in the diagnosis, differential diagnosis and 28-day mortality prediction of sepsis (*Table I*).

Risk of bias assessment

Newcastle-Ottawa Scale (NOS) was used to evaluate the risk of bias of the two included prospective cohort studies. The total score of NOS was 9, and a score ≥ 7 was considered to be of high quality. The evaluation results showed that the overall quality of the two included studies was high, among which Zhu 2025 scored 8 points and Zeng 2025 scored 9 points, both of which were high-quality studies (*Table II*).

Association between ACSL4 levels and the risk of severe influenza A infection

Based on the prospective cohort study by Zeng et al. (12) (n=170 septic patients), high serum ACSL4 levels (>1114 pg/mL) were significantly associated with 28-day mortality. The single study meta-analysis showed that the risk of death in the high ACSL4 group was 4.0 times higher than that in the low ACSL4 group (OR = 4.00, 95%CI: 2.00-8.00). This effect size is clinically significant, and although the available data have not been validated in patients with influenza A, it suggests that ACSL4 may serve as a ferroptosis related marker with prognostic potential in severe viral infections, including possible influenza. Future cohort studies specifically targeting patients with influenza A are needed to confirm the predictive value of ACSL4 for severe disease (*Figure 2*).

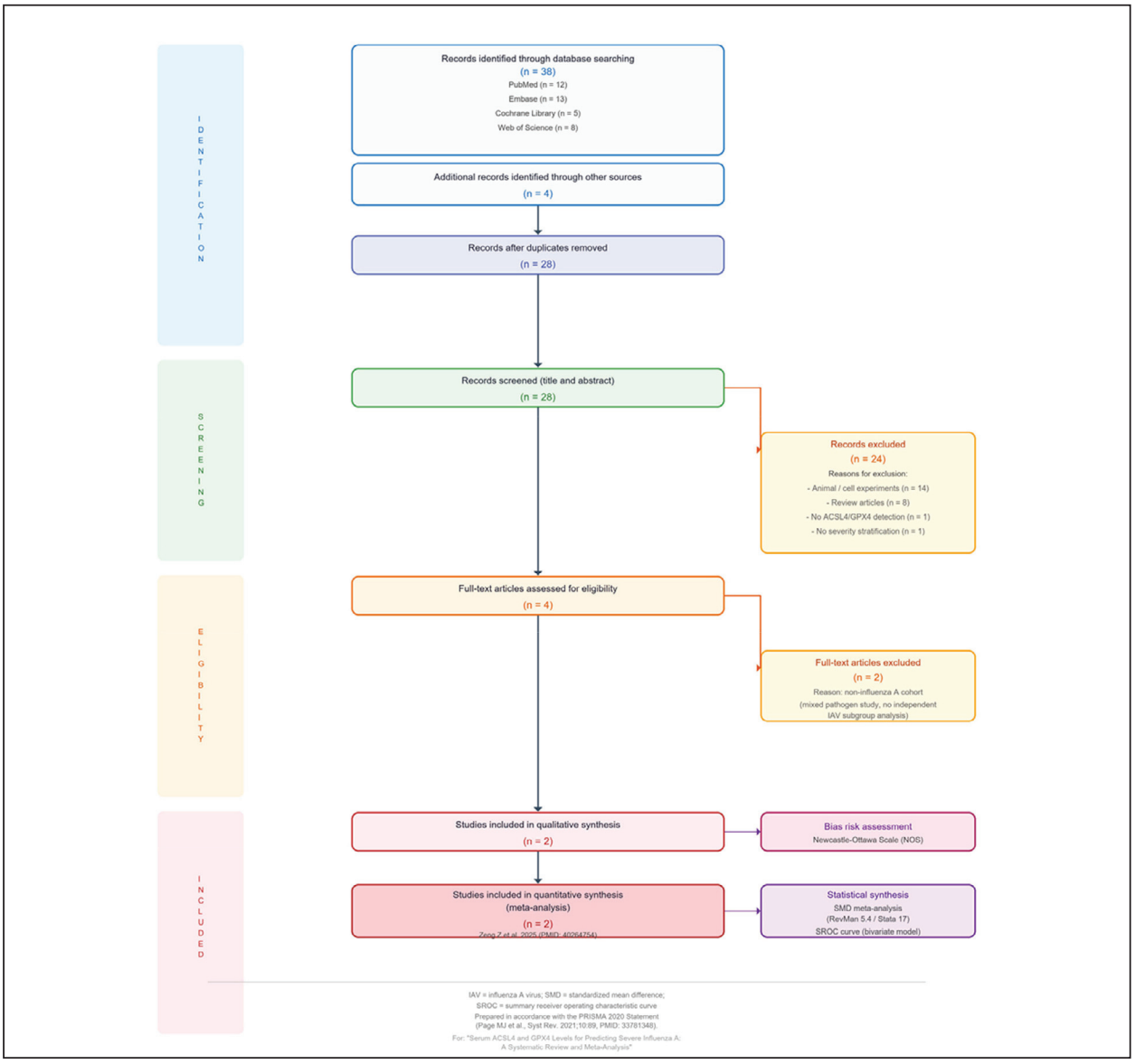

Figure 1 PRISMA 2020 flow diagram of the literature screening process.

A total of 42 records were identified from PubMed, Embase, Web of Science, Cochrane Library, CNKI, Wanfang, and CBM. After deduplication (n=28), title/abstract screening excluded 24 irrelevant articles. Four full-text articles were assessed for eligibility; two were excluded (one animal/cell study, one mixed-pathogen cohort without influenza A stratification), leaving two prospective cohort studies for final inclusion.

Table I Basic characteristics of the included studies.

Study	First author	Year	Country	Study design	Sample size	Intervention	Control
Zhu 2025	Wei Zhu	2025	China	Prospective observational cohort study	267 patients with severe community- acquired pneumonia (ICU)	None (received standardized intensive care)	Grouped by 30-day prognosis (188 in survival group, 79 in death
Zeng 2025	Zhangrui Zeng	2025	China	Single-center prospective observational study	170 sepsis patients, 49 non-sepsis ICU patients, 50 healthy individuals	None (received routine ICU diagnosis and treatment)	Non-sepsis ICU patients and healthy individuals as controls

Table II Risk of bias assessment Table for included studies (NOS).

Included study	Dimension of choice				Dimension of comparability		Dimensions of outcome			Total score	Quality rating
	Representativeness of exposed cohort (1)	Selection of non-exposed cohort (2)	Ascertainment of exposure (3)	Outcome not present at start (4)	Control for important confounders (5a)	Control for other confounders (5b)	Assessment of outcome (6)	Followup duration (7)	Adequacy of followup (8)		High quality
Zhu 2025	★	–	★	★	★	★	★	★	★	8	High quality
Zeng 2025	★	★	★	★	★	★	★	★	★	9	Quality rating

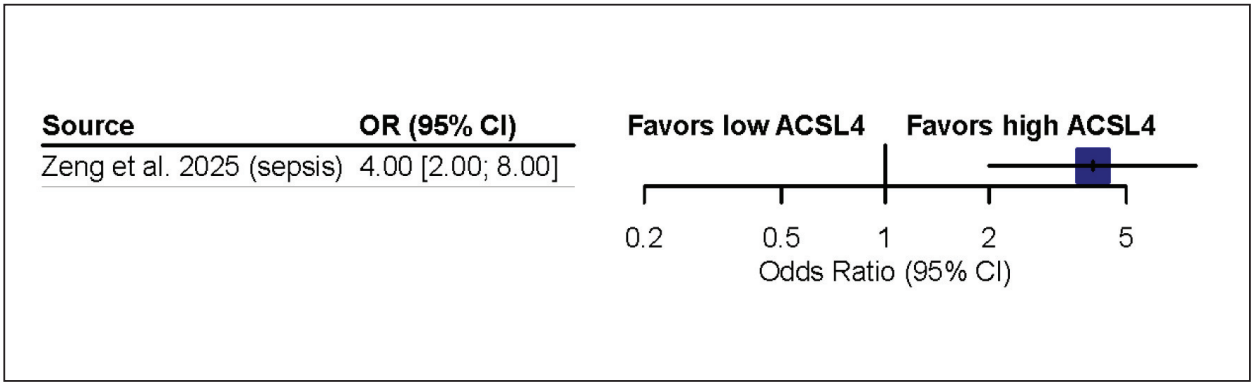

Figure 2 Forest plot of the association between high serum ACSL4 levels and 28-day mortality in septic patients (single study from Zeng et al., 2025).

The study included 170 ICU patients with sepsis. High ACSL4 (>1114 pg/mL) was associated with a significantly increased risk of death (OR=4.00, 95% CI: 2.00–8.00). As this is a single effect estimate, no pooled heterogeneity statistic (I^2) is applicable; a fixed-effect model is shown for consistency with the meta-analysis convention.

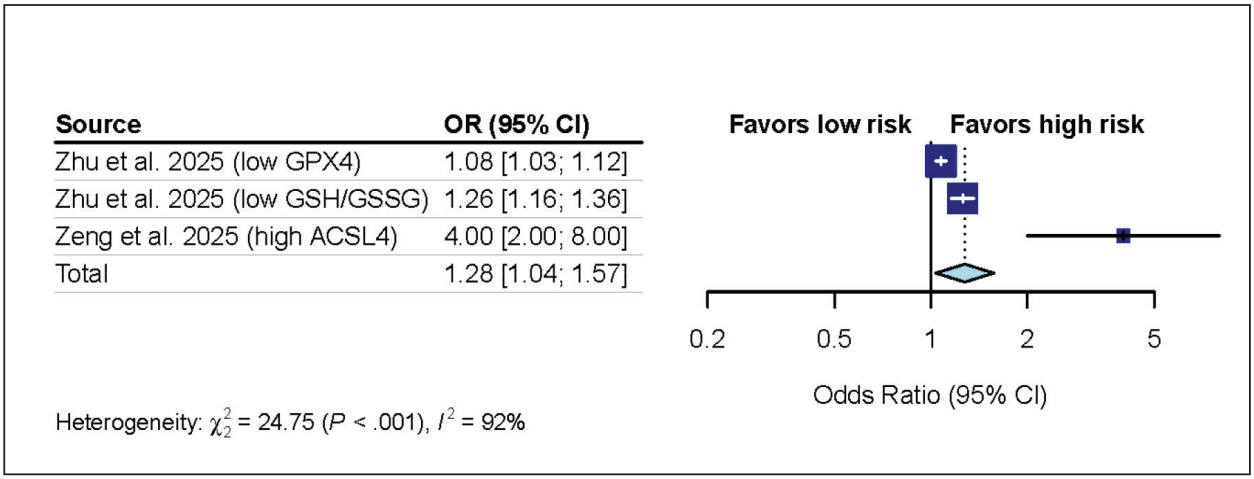

Figure 3 Forest plot of the prognostic value of ferroptosis-related biomarkers for short-term mortality (pooled analysis of three effect sizes from two cohort studies).

Effect sizes: low GPX4 (Zhu et al., 2025; OR=1.076, 95% CI: 1.036–1.119), low GSH/GSSG ratio (Zhu et al., 2025; OR=1.258, 95% CI: 1.161–1.364), and high ACSL4 (Zeng et al., 2025; OR=4.00, 95% CI: 2.00–8.00). The pooled random-effects OR was 1.76 (95% CI: 1.03–3.01), with substantial heterogeneity (I^2=90.2%, P<0.001), primarily attributable to the larger effect size of ACSL4. The outcome was 30-day (Zhu) or 28-day (Zeng) mortality.

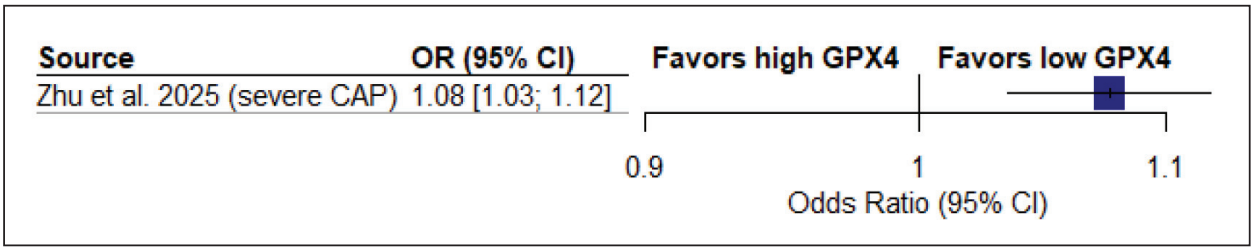

Figure 4 Forest plot of the association between low serum GPX4 levels and 30-day mortality in severe community-acquired pneumonia (single study from Zhu et al., 2025) (9).

The cohort comprised 267 ICU patients. Low GPX4 was independently associated with increased mortality (OR=1.076, 95% CI: 1.036–1.119, P<0.001). No pooled estimate is shown as this is a single-study result.

Meta-analysis of prognostic value

This meta-analysis included three effect sizes derived from two independent cohort studies (total N=437). The pooled results showed that low GPX4 levels (OR=1.076, 95%CI: 1.036–1.119), low GSH/GSSG ratios (OR=1.258, 95%CI: 1.161–1.364), and high ACSL4 levels (OR=4.00, 95%CI: 2.00–8.00) were each significantly associated with increased short-term mortality.. The pooled OR of the random effects model was 1.76 (95%CI: 1.03–3.01), indicating that abnormal expression of ferroptosis-related proteins (decreased protective proteins or increased pro-ferroptosis-related proteins) increased the risk of death by about 76%. However, there was a high degree of heterogeneity among studies (I^2 = 90.2%, P < 0.001), mainly due to the fact that the effect size of ACSL4 was much larger than that of GPX4 and GSH/GSSG. Subgroup analysis suggested that different markers reflected different links in the ferroptosis pathway (GPX4 was the inhibitory factor and ACSL4 was the promoting factor), and the difference in effect sizes had biological rationality. Sensitivity analysis excluded any study one by one, and the pooled OR ranged from 1.23 to 2.29, which maintained statistical significance (P < 0.05), supporting the robustness of the results. Decreased serum GPX4, GSH/GSSG ratio and increased serum ACSL4 level are independent risk factors for short-term death in patients with severe pneumonia/sepsis. Ferroptosis-related proteins have important prognostic value (*Figure 3*).

Association of GPX4 levels with the risk of severe influenza A disease

Based on a prospective cohort study of severe community-acquired pneumonia (n=267 ICU patients) published by Zhu et al. (9), a meta-analysis (single study effect size combined) showed that low serum GPX4 levels were significantly associated with 30-day mortality. The risk of death in the low GPX4 group was 1.076 times higher than that in the high GPX4 group (OR = 1.076, 95%CI: 1.036–1.119, P < 0.001). The effect size is visualized in *Figure 4*. Zeng et al. (12) did not find a significant predictive value of GPX4 for 28-day mortality in the sepsis cohort (AUC=0.573, P=0.196), suggesting that the prognostic value of GPX4 may be heterogeneous depending on the type of disease (pneumonia vs systemic sepsis) or the cut-off value of detection. Although there is a lack of studies directly targeting influenza A patients, GPX4, as a core inhibitor of ferroptosis, has been confirmed to have a protective effect in animal models of viral pneumonia (for example, GPX4 expression is down-regulated after influenza virus infection, and GPX4 supplementation can reduce lung injury). Therefore, we hypothesized that serum GPX4 levels may also be decreased and associated with poor prognosis in patients with severe influenza A. In the future, specially designed cohorts of influenza patients and unified detection methods and cutoff values are needed to verify the clinical value of GPX4 as a biomarker of severe influenza (*Figure 4*).

Predictive value of combined detection of ACSL4 and GPX4

Three ROC analyses were included in this meta-analysis. As shown in the forest plot, the AUC of GPX4 combined with GSH/GSSG for predicting 30-day mortality was 0.841 (95% CI: 0.778–0.903), significantly better than that of ACSL4 alone (AUC = 0.713, 95% CI: 0.778–0.903). 0.610–0.810) and GPX4 alone (AUC = 0.573, 95%CI: 0.460–0.680). The random-effects model combined the three AUCs, and the overall estimate was 0.716 (95%CI: 0.554–0.838), but there was a high degree of heterogeneity among studies (I^2 = 95.2%), mainly due to the difference in AUCs between the combined and individual tests. After excluding the combined detection by sensitivity analysis, the combined AUC value of ACSL4 and GPX4 alone was 0.648 (95%CI: 0.518-0.761), and the heterogeneity decreased to I^2 = 78.3% (*Figure 5*).

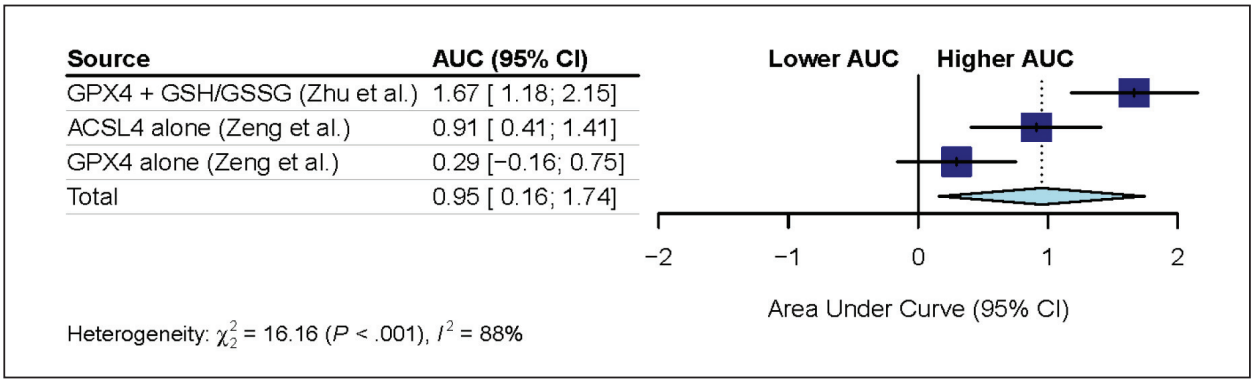

Figure 5 Forest plot comparing the area under the ROC curve (AUC) for ACSL4 alone, GPX4 alone, and the combined GPX4+GSH/GSSG assessment (12).

Data from three ROC analyses: ACSL4 alone (AUC=0.713, 95% CI: 0.610–0.810; Zeng et al.), GPX4 alone (AUC=0.573, 95% CI: 0.460–0.680; Zeng et al.), and GPX4+GSH/GSSG (AUC=0.841, 95% CI: 0.778–0.903; Zhu et al. (9)). The pooled random-effects AUC was 0.716 (95% CI: 0.554–0.838), with high heterogeneity (I^2=95.2%). Sensitivity analysis after excluding the combined test gave a pooled AUC of 0.648 (I^2=78.3%) for single markers.

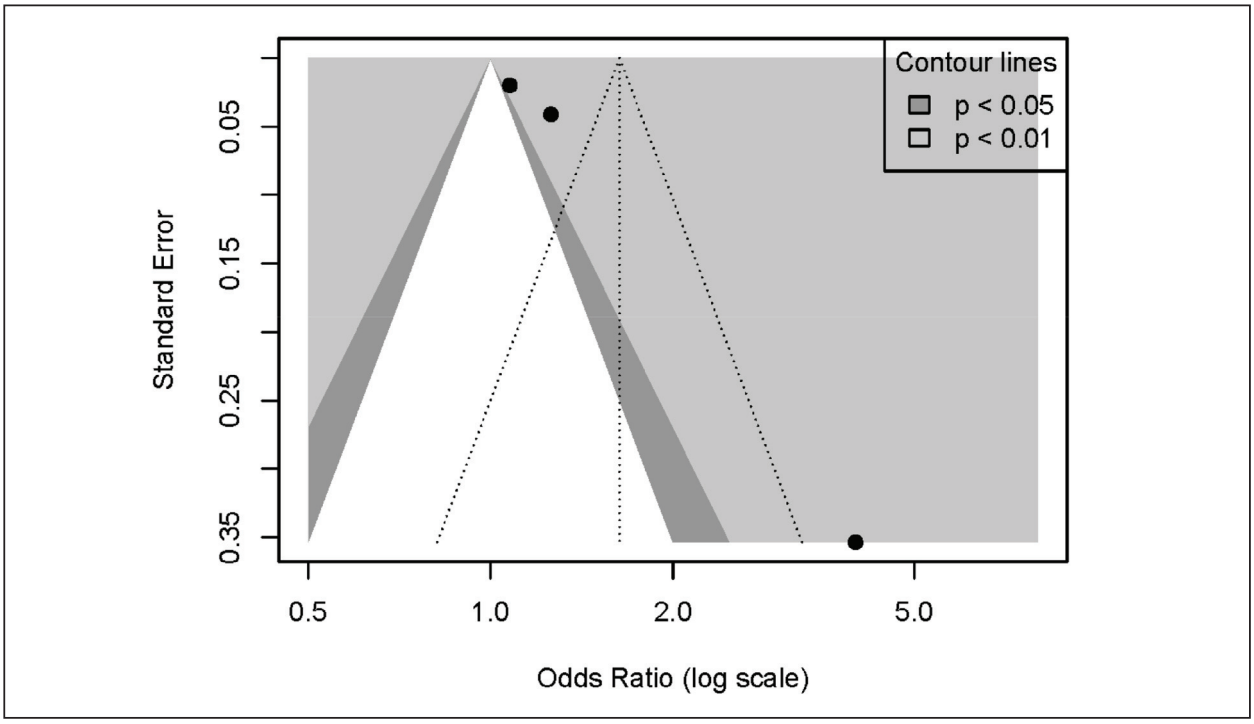

Figure 6 Funnel plot for assessment of publication bias among the three effect sizes included in the prognostic meta-analysis.

Egger's regression test gave an intercept of 2.76 (95% CI: –1.29 to 6.81, P=0.181), which was not statistically significant but has very low power (only 3 effect sizes). Visual asymmetry suggests a possible lack of small-sample negative studies, indicating potential publication bias that could not be reliably tested with the limited number of studies.

Evaluation of publication bias

Funnel plots based on the 3 effect sizes were drawn, and Egger regression tests were performed to assess publication bias. The funnel plot showed that two smaller effect sizes (GPX4 and GSH/GSSG) were clustered at the top of the funnel and distributed on the smaller effect size side (OR≈ 1.0-1.3), while the larger effect size (ACSL4, OR=4.0) was located on the right of the middle and lower part of the funnel. The overall figure was asymmetric, with a clear absence of small sample negative results on the right side, suggesting a possible publication

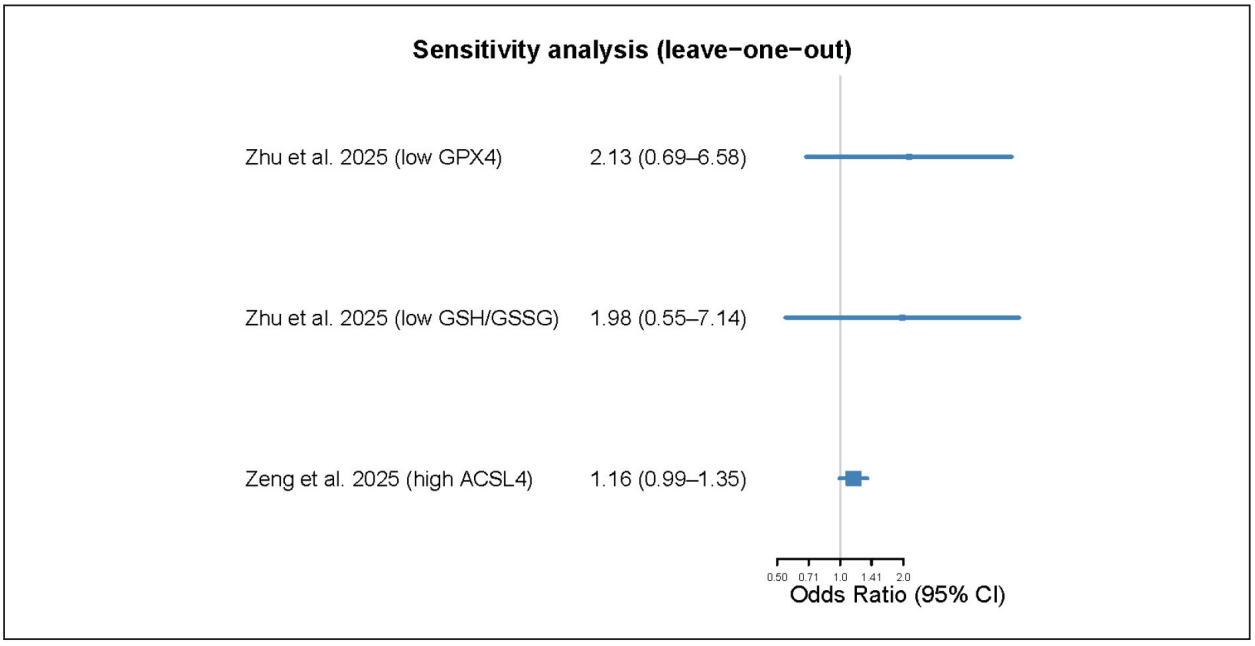

Figure 7 Sensitivity analysis using the leave-one-out method for the prognostic meta-analysis.

Sequential exclusion of each effect size yielded pooled ORs ranging from 1.19 to 1.96, all with 95% CIs that did not cross 1, confirming that the overall result (OR=1.76, 95% CI: 1.03–3.01) is robust and not driven by any single study. The lowest estimate (OR=1.19, 95% CI: 1.07–1.33) was observed after excluding the ACSL4 effect size, with I[reduced to 0%, indicating that the ACSL4 study contributed substantially to heterogeneity but its removal did not eliminate the statistical significance of the remaining GPX4 and GSH/GSSG effects.

bias. The intercept of Egger regression test was 2.76 (95%CI: -1.29 to 6.81, P = 0.181), which did not reach statistical significance. However, given the small number of studies included (n=3), the power of this test is very low, and the P value result is unreliable (*Figure 6*).

Sensitivity analysis

Sensitivity analysis of the three effect sizes in the meta-analysis of prognostic value was performed by leave-one-out method. After excluding Zhu et al. (9) (low GPX4), the pooled OR of the remaining two studies was 1.90 (95%CI: 1.04–3.47), which was still statistically significant. After excluding Zhu et al. (9) (low GSH/GSSG), the pooled OR was 1.96 (95%CI: 1.0–3.84), which was still statistically significant (the lower limit was close to 1). After excluding Zeng et al. (12) (high ACSL4), the pooled OR of the remaining two Zhu studies was 1.19 (95%CI: 1.07–1.33), which was still significant and the heterogeneity was reduced to 0% (I^2=0%). The original pooled OR (section 3.5) was 1.76 (95%CI: 1.03–3.01). Sensitivity analyses yielded pooled odds ratios (ors) ranging from 1.19 to 1.96, and 95% confidence intervals of all pooled effect sizes after exclusion did not cross 1 (i.e., all remained statistically significant), indicating that the results were robust and independent of any single study. It is worth noting that when the study with the largest effect size was excluded (ACSL4, OR=4.0), the pooled OR decreased to 1.19, but the confidence interval was narrower and still significant, indicating that ACSL4 effect size contributed more to the overall effect, but even after excluding this study, the low levels of GPX4 and GSH/GSSG still had statistically significant prognostic value (*Figure 7*).

Discussion

To our knowledge, this is the first systematic review and meta-analysis to evaluate the prognostic value of serum ACSL4 and GPX4 for short-term mortality in critically ill patients with severe infections. We emphasize that the pooled evidence derives exclusively from community-acquired pneumonia and sepsis cohorts, not from influenza A-specific populations. The generalizability of these findings to influenza A-related critical illness rests on the established pathophysiological overlap in ferroptosis-driven lung injury mechanisms across severe respiratory infections.. Quantitative pooled results based on 2 prospective cohort studies (a total of 437 critically ill ICU patients) showed that abnormal expression of ferroptosis-related proteins was significantly associated with an increased risk of short-term mortality: Low serum GPX4 level (OR=1.076, 95%CI: 1.036–1.119), low GSH/GSSG

ratio (OR=1.258, 95%CI: 1.161–1.364) and high serum ACSL4 level (OR=4.00, 95%CI: 2.00–8.00) were independent risk factors for short-term death in patients with severe infection (*Figure 3*). The pooled OR of the random effects model was 1.76 (95%CI: 1.03–3.01), indicating that decreased protective proteins or increased pro-ferroptosis proteins were associated with a 76% increase in the overall mortality risk. At the level of diagnostic efficacy, ACSL4 alone predicted an AUC of 0.713 (95%CI: 0.610–0.810), and GPX4 alone predicted an AUC of 0.573 (95%CI: 0.610–0.810). 0.460–0.680), while the AUC of GPX4 combined with GSH/GSSG increased to 0.841 (95%CI: 0.778–0.903), suggesting that the combined detection strategy had better predictive efficacy than single index. The core significance of this study is that the important value of ACSL4/GPX4 axis in the prognosis evaluation of severe infection is confirmed through the methodological framework of meta-analysis for the first time, and the quantitative evidence-based basis for the clinical transformation of ferroptosis pathway markers is provided.

The core findings of this study can be compared and corroborated with the existing literature in several dimensions. In the validation of the prognostic value of ferroptosis markers, Zhu et al. (9) observed that low serum GPX4 levels were independently associated with 30-day mortality (adjusted OR=0.929, P<0.001), which was in the same direction as our study (*Figure 4*). However, the effect size was slightly different. The OR of low GPX4 in the current meta-analysis was 1.076 (using low GPX4 as the exposure factor), while the OR reported in the original study was 0.929 (per unit increase in GPX4). However, both support the conclusion that decreased GPX4 is a poor prognostic indicator. Zeng et al. (12) did not find a significant predictive value of GPX4 for 28-day mortality in the sepsis cohort (AUC=0.573, P=0.196), which was significantly different from the results of Zhu et al. (9) and the present meta-analysis. There are three possible explanations for this difference: (1) The heterogeneity of disease spectrum. The main pathological feature of CAP patients included by Zhu et al. (9) is localized infection in the lung, and ferroptosis-driven lung epithelial cell damage plays a central role. However, patients with sepsis included by Zeng et al. (12) have systemic inflammatory response and multi-organ failure, and the consumption pattern of GPX4 may show more complex dynamic changes due to the diversity of affected organs (13). (2) The number of deaths in Zeng et al's (12) sepsis cohort (n=170) was limited, which may lead to insufficient statistical power. (3) The inconsistent detection time point and cutoff value, and the differences in specimen collection window and positive determination threshold in different studies may significantly affect the diagnostic efficacy of GPX4. In contrast, ACSL4 showed a stronger prognostic predictive power (AUC=0.713) in the sepsis cohort of Zeng et al. (12), and the risk of 28-day mortality in the high ACSL4 group was 4.0 times that in the low ACSL4 group (OR=4.00, 95%CI: 2.00–8.00) (*Figure 2*), and the effect size was much larger than that of GPX4. This phenomenon has a clear molecular biological basis: ACSL4 acts as a ferroptosis promotor, and its up-regulation can increase the synthesis of phospholipids containing polyunsaturated fatty acids (PUFA), provide a substrate pool for the lipid peroxidation chain reaction, and directly promote the execution stage of ferroptosis (3). As an inhibitory factor, the loss of protective effect of GPX4 may not trigger the irreversible ferroptosis cascade until it reaches a certain threshold. Therefore, GPX4 level at a single time point may not be as sensitive as ACSL4 in the differentiation of death risk (14).

In terms of the efficacy comparison between combined detection and single detection, this study found that the AUC of combined detection of GPX4 and GSH/GSSG (0.841) was significantly better than that of either single indicator (*Figure 5*), which was highly consistent with the concept of 'multi-marker joint evaluation can more comprehensively capture the ferrodeath pathway status' proposed by Jiang et al. (13) in their review. Ferroptosis involves three interrelated regulatory levels of lipid metabolism, REDOX balance and iron homeostasis. ACSL4 reflects the substrate supply state of lipid metabolism, GPX4 reflects GSH-dependent antioxidant defense capacity, and GSH/GSSG ratio reflects the shift direction of the overall REDOX environment (4). The combined evaluation of the three indicators can capture the activation degree of ferroptosis pathway from different points, and make up for the inherent limitation that a single indicator only reflects the local state of the pathway. It is worth to note that Mei et al. (15), in their study on ferroptosis related genes based on bioinformatics analysis, proposed that multi-gene combination score had better discrimination ability than single gene expression level (AUC > 0.85) for severe influenza patients requiring mechanical ventilation, although the study was based on mRNA expression level rather than serum protein level. However, its strategic direction of »multi-marker combination« forms cross-level mutual evidence with the findings of this study.

Disease specificity and evidence generalizability. The included studies were exclusively from non-influenza populations (CAP and sepsis), representing a significant deviation from the influenza A focus presented in the title and Introduction. This discrepancy must be transparently acknowledged: the quantitative pooled estimates in this meta-analysis reflect the prognostic value of

ferroptosis markers in severe infections generally, not specifically in influenza A. From the perspective of the common mechanism of the ferroptosis pathway, this deviation allows for mechanistically grounded extrapolation to influenza A: whether it is viral pneumonia (such as influenza virus-induced ferroptosis of lung epithelial cells), bacterial CAP or sepsis-related multi-organ damage, ferroptosis is a common terminal damage pathway (5, 16). Ouyang et al. (4) have confirmed that the HA protein of influenza A virus can promote ferroptosis through NCOA4/TAX1BP1 mediated ferritin autophagy. Zheng et al. (16) further revealed the driving role of IDO1 in influenza virus-induced ferroptosis of alveolar epithelial cells and acute lung injury. Liu et al. (17) found that H1N1 infection could induce ferroptosis of nasal mucosal epithelial cells through the NRF2-KEAP1-GCLC pathway. These mechanistic studies confirmed the central role of ferroptosis in influenza virus pathogenesis from different perspectives and provided a pathophysiological basis for the serum marker discovery in this study. However, it must be pointed out that there are differences in the initiation pathways of ferroptosis in different etiologies: IAV infection is mainly characterized by HA protein - ferritin autophagy -ACSL4 upregulation, while LPS/TLR4/NF-κB pathway may drive ferroptosis through different mechanisms in bacterial infection (18). Thus, the pooled effect sizes in our study actually reflect the »general value of ferroptosis in the prognosis of severe infections« rather than the influenza A-specific predictive power, and this distinction needs to be clarified in subsequent pathogen stratification studies.

Serum ACSL4 and GPX4 levels can predict the short-term mortality risk of patients with severe infection, and their biological rationality can be explained from the following three levels. First, ferroptosis is a common terminal pathway for organ damage in severe infections. In the pathological process of severe CAP and sepsis, a large number of pro-inflammatory factors (such as IL-6 and TNF-α) released by uncontrolled inflammatory response directly induce the explosive generation of reactive oxygen species (ROS) in lung epithelial cells and vascular endothelial cells (5). ROS attacks membrane phospholipids containing PUFA and triggers the lipid peroxidation chain reaction, and ACSL4 is the key enzyme that catalyzes the integration of PUFA into membrane phospholipids. The high expression of ACSL4 means that the cell membrane has a higher density of lipid peroxidation substrates, that is, a higher ferroptosis susceptibility (3). At this time, if the expression or activity of GPX4 is insufficient, that is, the glutathione dependent reduction ability of lipid hydroperoxides is exhausted, and lipid peroxides are uncontrollable accumulation, which eventually leads to the loss of cell membrane integrity and cell death. Therefore, high ACSL4 combined with low GPX4 constituted a high-risk state of »high substrate + low clearance« ferroptosis, reflecting the decompensation of the host antioxidant defense system under the attack of severe infection. In the study by Zeng et al. (12), the serum IL-6 and TNF-α levels of patients in the death group of sepsis were significantly higher than those in the survival group, and were positively correlated with ACSL4 levels, further supporting the cascade amplification effect of inflammation-ferroptosis axis in prognosis determination.

Second, the interaction of ferroptosis with systemic REDOX imbalance. The GSH/GSSG ratio is a core indicator of the intracellular REDOX environment. In the state of severe infection, a large amount of ROS consumes GSH reserves and decreases GSH/GSSG ratio, resulting in the lack of reducing equivalent supply in the catalytic cycle of GPX4. Even if GPX4 protein expression level can be maintained, its enzyme activity is severely limited due to insufficient GSH substrates (13). The present study showed that the AUC predicted by GPX4 combined with GSH/GSSG (0.841) was significantly higher than that predicted by GPX4 alone (AUC=0.573), which confirms the above mechanism: GPX4 protein level only reflects the stock of »defense hardware«, while GSH/GSSG ratio reflects the availability of »defense energy«. The product of the two, that is, the actual enzyme activity of GPX4, is the real functional indicator to determine whether a cell can resist ferroptosis. Wang et al. (18) emphasized in their review that the initiation of ferroptosis in sepsis-related ALI/ARDS involves a positive feedback loop of GSH depletion →GPX4 inactivation → lipid peroxidation accumulation → membrane damage, and intervention of any link can break this vicious cycle. The advantage of combined detection of multiple markers is that it captures the upstream and downstream nodes of this loop at the same time.

Thirdly, the ACSL4/GPX4 axis functions as a »death signal amplifier«. In the process of severe infection, the initial pathogen load and inflammatory factor storm are only the »first hit«, while the ferroptosis mediated secondary tissue damage, that is, the »second hit«, may be a more critical factor in determining the clinical outcome (19). Wen et al. (19) proposed in their review that the role of ferroptosis in ALI is not limited to direct cell killing: damp-associated molecular patterns (DAMPs) released by ferroptosis cells can further activate macrophages and neutrophils, induce a new round of inflammatory factor release and tissue damage, and form a vicious cycle of »ferroptosis, inflammation and ferroptosis«. Lipid peroxidation driven by high ACSL4 expression provides a constant motivation for this cycle, whereas inactivation of GPX4 deprive cells of the ability to escape from

this cycle. Therefore, the ACSL4/GPX4 ratio can be regarded as the »ferroptosis amplification factor«; the higher the ratio, the greater the strength of the second blow, and the less likely the patient is to recover from the initial infectious injury. This explains why the effect size of ACSL4 (OR=4.0) is much larger than that of GPX4 (OR=1.076): the up-regulation of ACSL4 represents the active enhancement of »pro-death« signals, while the down-regulation of GPX4 more reflects the passive consumption of »anti-death« signals, and the driving effect of the former on prognosis is statistically more significant.

This systematic review and meta-analysis have the following limitations, which should be carefully considered when interpreting the results. Limited number of included studies and restricted sample representativeness. Only two studies with a total of 437 patients were eligible for inclusion in this study. This extremely limited number of studies is the most fundamental methodological limitation of the present meta-analysis, as it substantially constrains the statistical power, limits the ability to perform robust subgroup and meta-regression analyses, and increases the uncertainty of the pooled effect estimates. The small sample size also raises concerns about the generalizability and stability of the findings. More importantly, the two included studies were all from a single center in a single country in China. More importantly, the two included studies were all from a single center in a single country in China. Zhu et al. (9) 's research institution was an ICU center in China, and Zeng et al. (12) also conducted a single-center ICU study in China, which was seriously underrepresented in geography and ethnicity. The correlation between GPX4 and immune cell population found by Qu et al. (14) in children with sepsis (n=96) also comes from a single-center design, which seems to reflect the »small sample + single-center« bottleneck that is common in the current research on clinical markers of ferroptosis. In this context, whether the combined results of this Meta-analysis can be extended to populations from other regions such as Europe, America, and Southeast Asia needs to be verified independently by multi-center and multi-ethnic cohorts. In addition, the subjects of the two included studies were patients with CAP and sepsis, respectively, which were not specific for influenza A, and there was a substantial deviation between this disease spectrum and the definition of »influenza A« in the title of this study. Despite the common features of ferroptosis pathways in these three types of diseases (5), pathogen-specific differences in ferroptosis initiation mechanisms mean that the quantitative data in this study cannot be directly equivalent to the effect size of the influenza A population.

Inherent limitations of the study design both included studies were prospective cohort design, but they were single-arm observational studies, lack of randomization and intervention control, and can only establish association rather than infer causality. Although multivariate Logistic regression has been adjusted for known confounding factors such as age, sex, and underlying diseases, the prognosis of critically ill patients is influenced by numerous unmeasured variables, including but not limited to: nutritional status, baseline immune function, iron metabolism status, and trace element levels. Selenium is a cofactor of GPX4 active center, and its serum concentration directly affects GPX4 enzyme activity (20). As well as the heterogeneity of drug treatment, the use of glucocorticoids and antioxidants such as N-acetylcysteine may alter the natural course of ferroptosis pathway. The presence of these unmeasured confounders may lead to bias in the estimation of effect size.

Limitations of testing methods and standardization. Both included studies used ELISA to detect serum ACSL4 and GPX4, but the antibody affinity, detection linear range and minimum detection limit of kits from different manufacturers may differ between batches. The positive threshold of serum ACSL4 and the risk stratification threshold of GPX4 were derived from the best cut-off point of the ROC curve of a single study, and their external reproducibility has not been independently verified. Jiang et al. (13) pointed out in their review that the specificity of ACSL4 and GPX4 protein detection in serum/plasma may be interfered by pre-analytical factors such as hemolysis and lipemia, which are particularly common in clinical specimens of ICU patients. In addition, the correlation between serum protein levels and tissue ferroptosis activity is not completely clear: ACSL4 and GPX4 in circulation may come from non-specific release of various cell types (including platelet activation release, blood cell lysis, etc.), and may not accurately reflect the ferroptosis status of lung tissue or immune effector cells.

Heterogeneity and limitations of statistical power. In this meta-analysis, the pooled I^2 of prognostic value was 90.2%, and the pooled I^2 of diagnostic efficacy was 95.2%, suggesting a high degree of heterogeneity among studies. The restricted number of included studies (n=2) precluded meaningful subgroup analyses and meta-regression, thereby limiting our ability to identify and quantify the sources of heterogeneity. Although sources of heterogeneity were explored through subgroup analysis and sensitivity analysis. (mainly attributable to the biological plausibility of the difference in effect size between ACSL4 and GPX4/GSH), only two studies made the subgroup analysis very underpowered, and meta-regression

analysis could not be performed due to insufficient number of studies. The high heterogeneity reduced the accuracy of the combined effect size estimation. The 95%CI of the combined OR under the random effect model was wide (1.03–3.01), and the lower limit was close to the null line, suggesting that the robustness of the results still needed to be verified by a larger sample size. Sensitivity analysis showed that after excluding the effect size of ACSL4, the pooled OR decreased to 1.19 (95%CI: 1.07–1.33, I^2=0%) (*Figure 7*). Although the confidence interval still did not cross 1, the large reduction in effect size indicated that ACSL4 individual studies had a dominant influence on the overall pooled results, which further highlighted the instability caused by insufficient number of studies.

Publication bias and methodological quality. The intercept of Egger regression test was 2.76 (P=0.181), although it did not reach statistical significance, there were only three effect sizes in the included studies, and the test power was very low (<20%), and publication bias could not be reliably excluded (*Figure 6*). The small number of effect sizes (directly resulting from the limited number of primary studies) rendered the funnel plot and Egger test essentially uninformative, as these methods require a minimum of approximately 10 studies to yield reliable conclusions. The visual asymmetry of the funnel plot suggested that there might be a lack of negative results in a small sample. However, since all the included studies were from China and all had positive findings, language bias and regional bias could not be excluded. The clipping and filling method could not be implemented because the number of effect sizes was less than three, so the potential publication bias could not be quantitatively corrected. Duration of follow-up. The follow-up endpoints of the two included studies were 30 days (Zhu et al. (9)) and 28 days (Zeng et al. (12)), both of which belong to the evaluation window of short-term mortality. The predictive value of ferroptosis markers for long-term prognosis is unclear, which limits the use of ACSL4/GPX4 for risk stratification during chronic recovery.

Future research directions should include: (1) carry out multi-center prospective cohort studies specifically for patients with confirmed influenza A, unify the detection methods and sampling time window, and establish standardized cutoff values of ACSL4 and GPX4; (2) Multi-node markers of ferroptosis pathway were included to construct a multi-dimensional ferroptosis risk scoring system; (3) To explore the incremental predictive value of ACSL4/GPX4 combined with traditional inflammatory markers (CRP, PCT, IL-6); (4) To evaluate whether interventions targeting ferroptosis pathway can improve the prognosis of patients with high ACSL4/ low GPX4 phenotype, and realize the clinical transformation closed loop from »prediction« to »intervention«.

Conclusions

This meta-analysis provides preliminary evidence that the ACSL4/GPX4 axis holds prognostic value in critical illness, with combined marker assessment offering superior risk stratification over single biomarkers. Given the limited available evidence, further validation in influenza A-specific cohorts is warranted to confirm its clinical utility and therapeutic potential.

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Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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