Angiopoietin-2 as a prognostic biomarker in septic adult patients: a systemic review and meta-analysis

Mengke Zhuo[1], Sifeng Fu[2], Yawen Chi[1], Xinghua Li[1], Sirui Li[1], Xiaochun Ma[1] and Xu Li[1*]

Abstract

Background The impairment of endothelial function represents a key pathophysiological mechanism in the development of sepsis. This research aimed to evaluate the prognostic significance of angiopoietin-2 (Ang-2), an endothelial biomarker, in predicting mortality in sepsis patients.

Methods Chinese and English studies were systematically retrieved in PubMed, Cochrane Library, Embase, WanFang, CNKI, CQVIP, and CBM databases up to July 16, 2023. We conducted a study selection established upon predefined inclusion and exclusion criteria and used the Newcastle-Ottawa scale (NOS) to assess its quality. We extracted available data from the included studies for data analysis.

Results The final inclusion comprised 33 studies with 4703 participants. According to the NOS, one study was of medium quality, while the rest were of high quality. In comparison to survivors, the levels of Ang-2 in non-survivors were markedly elevated [standardized mean difference (SMD) = 1.08, 95% confidence intervals (CI) 0.68–1.49, $P < 0.001$], and the same results were also observed in the subgroup that met sepsis 3.0 diagnosis criteria (SMD = 0.63, 95% CI 0.11–1.14, $P = 0.017$). The results comparing Ang-2 levels between non-survivors and survivors were independent of duration of follow-up, sample sources, type of study, and region. Ang-2 was a risk factor for mortality [odds ratios (OR) = 1.16, 95% CI 1.09–1.23, $P < 0.001$]. Ang-2 was demonstrated to be able to predict mortality in septic adult patients [area under the curve (AUC) = 0.76, 95% CI 0.70–0.82, $P < 0.001$].

Conclusions Ang-2 level was positively correlated with risk of death in sepsis patients. Ang-2 might be a useful and valuable biomarker for predicting mortality in septic adult patients.

Keywords Angiopoietin-2, Sepsis, Biomarkers, Mortality, Meta-analysis

*Correspondence:
Xu Li
vincentzh002@outlook.com
[1]Department of Critical Care Medicine, The First Affiliated Hospital of China Medical University, North Nanjing Street 155, Shenyang, Liaoning Province 110001, China
[2]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Institute of Respiratory Health, State Key Laboratory of Respiratory Diseases, Guangzhou 510120, China

Introduction

Sepsis is fatal organ dysfunction due to an uncontrolled immune response to infection [1]. Although the Surviving Sepsis Campaign (SSC) guideline has acknowledged the potential significance of biomarkers in the treatment and prediction of the prognosis of sepsis, it fails to provide recommendations for the use of any biomarker for predicting sepsis prognosis [2]. Due to the intricate interplay of procoagulant, pro-inflammatory, and anti-inflammatory mechanisms that underlie the pathophysiology, identifying the ideal biomarker for sepsis is a challenge. Endothelial cells, which line the luminal surface of all blood vessels, serve as the first barrier separating blood from organs. Therefore, endothelial cells are the first sites to be invaded by pathogens. Endothelial activation or dysfunction is recognized as one of the main pathophysiological mechanisms and directly contributes to the morbidity and mortality of sepsis [3, 4].

Angiopoietin-2 (Ang-2), a growth factor secreted by endothelial cells in response to noxious or inflammatory stimuli, is associated with increased vascular permeability. It has gained much attention as one of the key regulators of microvascular endothelial function over the past two decades [5, 6]. Ang-2 has dual potential in clinical practice: as a prognostic biomarker and as a therapeutic target for conditions characterized by vascular leakage [7, 8]. Wollborn et al. conducted research on patients after cardiac surgery and discovered that Ang-2 was associated with capillary leak and could predict complications after cardiac surgery [7]. In another study, Ang-2 levels were higher in patients with Capillary Leak Syndrome (CLS) compared to those without CLS at various time points, and 30-day mortality was higher in CLS patients [8].

Ang-2 has been extensively studied in the context of sepsis. The function of Ang-2 in sepsis is intricate, exhibiting both beneficial and detrimental effects. In addition to its established role in angiogenesis, it also helps optimize the control of local infections, such as recruiting inflammatory cells and inducing appropriate permeability to facilitate migration [9]. The effects of Ang-2 may become harmful whenever this initial local response transforms into a systemic condition characterized by a widespread and overwhelming immune response [10]. A meta-analysis by Pregernig et al. [11] revealed that in adult sepsis patients, non-survivors exhibited elevated levels of Ang-2 compared to survivors. However, with the updated definition of sepsis, the conclusions of relevant studies were inconsistent [12–14]. Therefore, this meta-analysis aims to assess the potential prognostic significance of Ang-2 in septic adult patients in a larger population and under sepsis 3.0 diagnostic criteria.

Methods

This research was executed following the protocols outlined in the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [15] (Supplementary Material 1).

Search strategy

PubMed, Cochrane Library, Embase, WanFang, CNKI, CQVIP, and CBM databases were last retrieved on 07/16/2023, and the subject terms were from PubMed's Medical Subject Headings (MeSH) and SinoMed's Chinese Medical Subject Headings (CMeSH). For example, in PubMed, the operators "AND" and "OR" are used to search for a combination of "Sepsis" [MeSH], "Septic shock" [MeSH], "Angiopoietin-2" [MeSH], "Prognosis" [MeSH], and the corresponding keywords. The search strategy underwent slight modification to meet the specific requirements of each individual database. The specific details of each database were provided in Supplementary Material 2. Furthermore, to identify potentially relevant articles, we thoroughly examined the bibliographies of the included articles.

Study inclusion and exclusion criteria

Two authors (MKZ, YWC) independently screened the abstracts and titles based on the following inclusion criteria and then retrieved and reviewed the full texts. Any discrepancies were resolved by consensus. Inclusion criteria: (1) observational study, and the language was limited to English or Chinese; (2) the study subjects were aged 18 years or older and had sepsis, including severe sepsis and septic shock; (3) Ang-2 as an indicator for predicting mortality in sepsis; (4) survival outcomes; (5) one of the following types of data could be obtained directly or indirectly from the study: mean Ang-2 concentrations in both the survivor and non-survivor cohorts, the hazard ratio (HR) or OR with its 95% CI to analyze the correlation between Ang-2 and mortality, and the AUC with its 95% CI to predict mortality. Exclusion criteria: (1) in vitro or animal studies; (2) non-original studies, such as reviews, conference abstracts, comments, letters to editors, etc.; (3) studies with duplicate subjects. For studies with replicate observations, we included more recent studies or studies that provided additional data.

Quality assessment

The quality measurement was carried out independently by two researchers (XHL, MKZ) using the NOS, and any inconsistencies were addressed in collaboration with an additional researcher (SRL). The NOS comprises three components: selection of the study population, intergroup comparability, and measurement of outcomes. The detailed criteria and their corresponding scores are outlined as mentioned below: the representation

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of the exposed cohort (1); selection of the unexposed cohort (1); identification of exposure variables (1); demonstration that absence of the anticipated outcomes at baseline (1); comparability between groups established upon research design or statistical analysis (2); outcome assessments were accurate and unbiased (1); adequate follow-up to achieve outcomes (1); cohort follow-up was adequate (1). Every "yes" answer was scored. According to the overall score, research with a score of 7–9 was categorized as high quality, 4–6 as medium quality, and 0–3 as low quality.

Data extraction
Two authors (MKZ, SFF) independently extracted the following data from the published studies and corresponding additional materials, subsequently recording them in Microsoft Excel. When there were discrepancies in the data, the two authors collaborated to ensure consistency. The data included the following: (1) study information: first author, year of publication, region, and study type; (2) demographic and clinical information: study background, setting, study size, diagnostic criteria for sepsis, disease severity, infection site, whether patients with immunodeficiency or a history of radiotherapy and chemotherapy for cancer were excluded, treatments, and follow-up duration. (3) Ang-2 testing: the time point and method; (4) results: the mean Ang-2 levels in the cohorts of survivors versus non-survivors, the HR or OR with its 95% CI for analyzing the correlation between Ang-2 and mortality, the AUC and its 95% CI for predicting mortality, and the mean Ang-2 levels of patients in the sepsis cohort across different disease severity groups. When extracting Ang-2 levels from two groups, we recorded the data at the earliest time point if there were multiple points of data. For the analysis of OR, multivariate adjusted values were extracted and analyzed. If the values of multivariate analysis are not available, the values of univariate analysis are utilized. For studies that did not report the required data for the analyses, we asked the authors for the necessary data.

Statistical analysis
All statistical analyses were performed utilizing Stata 14.0. We mainly assessed the relationship between Ang-2 measurements and mortality. Outcomes were assessed using SMD, OR, AUC, and their respective 95% CI. The mean and standard deviation (SD) values were essential for calculating SMD. For continuous variables reported as median (interquartile range) or median (minimum to maximum value), the mean with SD was estimated using the methods reported by McGrath et al. [16]. If studies reported standard errors of the mean (SEM) instead of SD, the SD was computed using the formula provided by the Cochrane Collaboration: $SD = SEM * \sqrt{(\text{sample size})}$

[17]. For effect sizes [(OR or HR) and AUC], the pooled effect size and 95% CI were calculated. If a study provided only HR, it was included in the analysis as equivalent to the OR value. In order to assess heterogeneity, the I^2 index was calculated, when the I^2 value is greater than 50%, heterogeneity is considered to be high. For the results showing significant heterogeneity and including more than 10 studies, we conducted subgroup analysis and sensitivity analysis in order to identify the origin of heterogeneity and evaluate the robustness of the outcomes. If the source of heterogeneity could not be identified, a random-effect model was applied to data analysis. When $I^2 \leq 50\%$, the heterogeneity was deemed acceptable and a fixed-effect model was adopted. Funnel plots and Egger's linear regression method were conducted to evaluate the publication bias in the included research. If necessary, the stability of the results was further assessed by the trim-and-fill method.
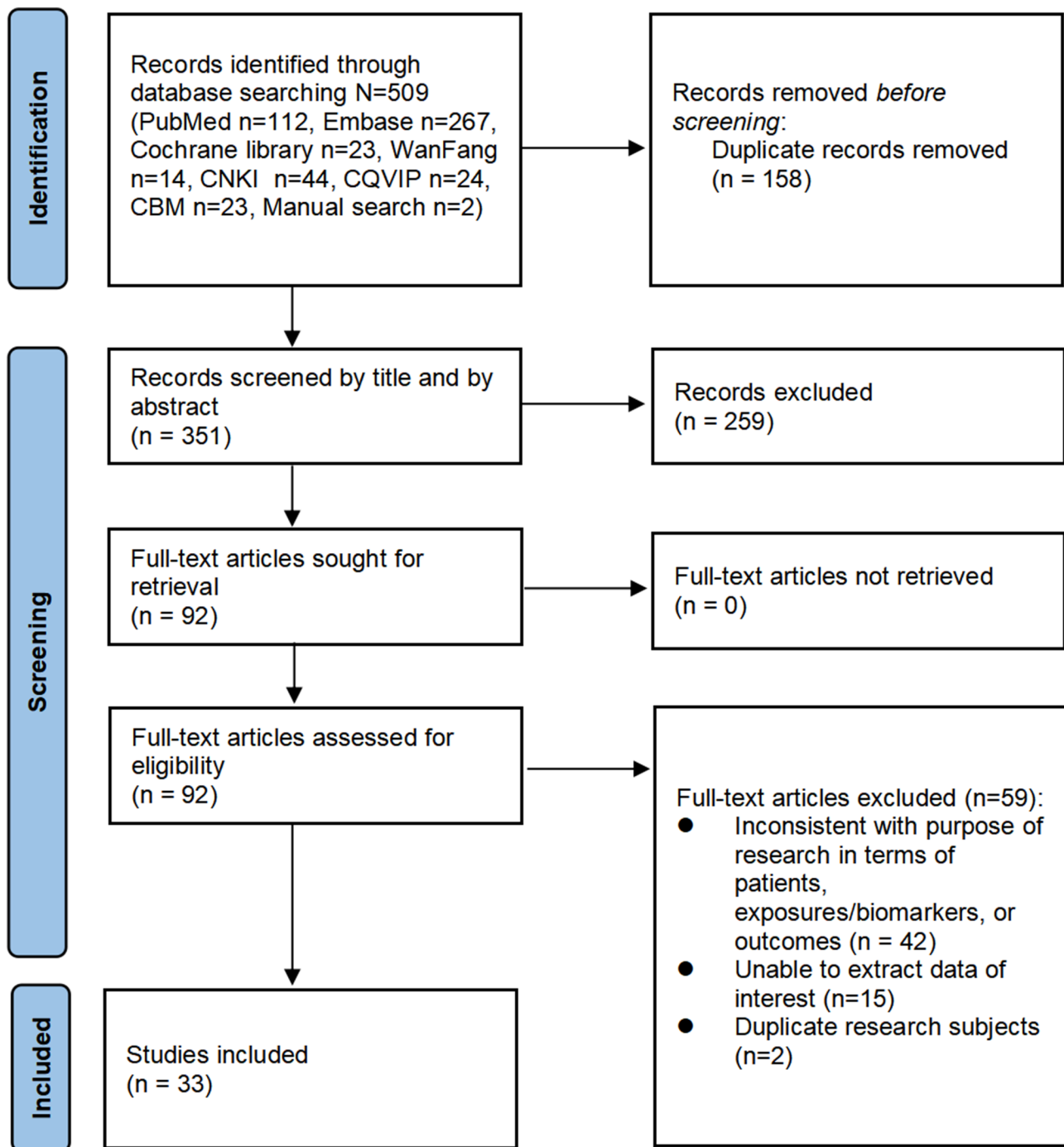
Results
Study selection and characteristics of included studies
A total of 509 articles were obtained through electronic and manual searches, with a final inclusion of 33 articles for the meta-analysis, including 23 English articles and 10 Chinese literatures. The process of research selection is shown in Fig. 1.

The basic features of the studies included are outlined in Table 1 and Supplementary Material 3. Of the 33 studies included, 16 used sepsis 3.0 diagnostic criteria, two studies did not specify diagnostic criteria. 17 studies measured plasma Ang-2 levels, while 16 studies measured serum Ang-2 levels. 25 studies described infection sites, mainly in the lungs, abdomen, and urinary system. Excluding two studies that did not specify the follow-up duration, 18 studies provided 28-day mortality, seven studies provided in-hospital mortality, five studies observed 30-day mortality, and the remaining one reported 90-day mortality. The overall methodological assessment of all included studies as evaluated by the NOS indicated a moderate to high level of quality (Supplementary Material 4).

Comparison of Ang-2 levels in survivors versus non-survivors
A total of 28 studies compared Ang-2 concentrations between survivors and non-survivors. Considering the significant heterogeneity in the results ($I^2 = 94.7\%$, $P < 0.001$), we employed a random-effect model and adopted SMD as the metric for evaluating the effects. The results showed that Ang-2 levels were significantly higher in non-survivors compared to survivors (SMD = 1.08, 95% CI 0.68–1.49, $P < 0.001$) (Fig. 2A). We conducted subgroup analyses to assess the effects of diagnostic criteria,

Identification

Records identified through database searching N=509 (PubMed n=112, Embase n=267, Cochrane library n=23, WanFang n=14, CNKI n=44, CQVIP n=24, CBM n=23, Manual search n=2)

Records removed *before screening*:
    Duplicate records removed (n = 158)

Screening

Records screened by title and by abstract (n = 351)

Records excluded (n = 259)

Full-text articles sought for retrieval (n = 92)

Full-text articles not retrieved (n = 0)

Full-text articles assessed for eligibility (n = 92)

Full-text articles excluded (n=59):
- Inconsistent with purpose of research in terms of patients, exposures/biomarkers, or outcomes (n = 42)
- Unable to extract data of interest (n=15)
- Duplicate research subjects (n=2)

Included

Studies included (n = 33)

Fig. 1 Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow chart

follow-up duration, sample sources, type of study, and region.

In 15 studies using sepsis 3.0 diagnostic criteria, Ang-2 levels were also significantly higher in non-survivors than in survivors (SMD=0.63, 95% CI 0.11–1.14, P=0.017). A high I^2 value of 93.3% (P<0.001) indicated significant statistical heterogeneity in this subgroup (Fig. 2C). In eight studies that used sepsis 1.0 diagnostic criteria, Ang-2

levels were also higher in the non-survivors than in survivors (SMD=1.16, 95% CI 0.55–1.77, P<0.001). A high I^2 value of 93.9% (P<0.001) indicated significant statistical heterogeneity in this subgroup (Fig. 2C). In two studies using sepsis 2.0 diagnostic criteria, there was no statistically significant difference in Ang-2 levels between survivors and non-survivors (SMD=1.25, 95% CI -1.54-4.03, P=0.380). A high I^2 value of 91.3% (P<0.001) indicated

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Table 1 Characteristics of all included studies

Author	Year	N	Region	Design	Definition	Severity	Source	NOS Score
Anderson [18]	2019	400	Non-Asia	MC, PC	Sepsis 3.0, sepsis 2.0	Sepsis, severe sepsis, septic shock	Plasma	8
Belli [13]	2022	35	Non-Asia	SC, PC	Sepsis 3.0	Septic shock	Plasma	9
Beurskens [12]	2020	21	Non-Asia	SC, PC	Sepsis 3.0	Sepsis, septic shock	Plasma	8
Davis [19]	2010	83	Non-Asia	SC, PC	Sepsis 1.0	Sepsis, severe sepsis	Plasma	7
Fang [20]	2015	440	Asia	SC, PC	Sepsis 2.0	SIRS, sepsis, severe sepsis, septic shock	Serum	7
Inkinen [21]	2019	619	Non-Asia	MC, RC	Sepsis 1.0	Sepsis, septic shock	Plasma	9
Karamouzos [14]	2021	128	Non-Asia	MC, RC	Sepsis 3.0	Sepsis	Plasma	9
Kazune [22]	2019	89	Non-Asia	MC, PC	Sepsis 3.0	Septic shock	Plasma	8
Kranidioti [23]	2009	90	Non-Asia	MC, PC	Sepsis 1.0	Sepsis, severe sepsis, septic shock	Serum	7
Palud [24]	2015	20	Non-Asia	SC, PC	Sepsis 2.0	Septic shock	Plasma	7
Ricciuto [25]	2011	70	Non-Asia	MC, PC	Modified sepsis 1.0	Severe sepsis	Plasma	8
Seol [26]	2020	145	Asia	SC, RC	Sepsis 3.0	Sepsis, septic shock	Plasma	8
Sexton [27]	2020	86	Non-Asia	SC, PC	Sepsis 3.0	Sepsis	Plasma	8
Siner [28]	2009	46	Non-Asia	SC, PC	Sepsis 1.0	Sepsis, severe sepsis, septic shock	Serum	7
Walborn [29]	2020	103	Non-Asia	MC, RC	Sepsis 1.0, sepsis 2.0	Sepsis	Plasma	7
Higgins [30]	2018	166	Non-Asia	MC, RC	Sepsis 1.0	Sepsis, severe sepsis, septic shock	Plasma	7
Lin [31]	2015	96	Asia	SC, PC	Sepsis 1.0	Severe sepsis	Plasma	8
Kümpers [32]	2009	21	Non-Asia	SC, PC	Not mention	Sepsis	Serum	6
Ma [33]	2020	41	Asia	SC, RC	Sepsis 3.0	Sepsis with ARDS	Plasma	7
Rosenberger [34]	2023	757	Non-Asia	SC, RC	Sepsis 1.0	Sepsis	Plasma	8
Statz [35]	2018	101	Non-Asia	MC, PC	Sepsis 3.0	Sepsis, septic shock	Plasma	8
Villar [36]	2021	232	Non-Asia	MC, PC	Sepsis 3.0	Sepsis	Serum	7
Parikh [37]	2006	22	Non-Asia	SC, PC	Sepsis 2.0	Sepsis, severe sepsis, septic shock	Serum	7
Chen [38]	2020	36	Asia	SC, PC	Sepsis 3.0	Sepsis, septic shock	Serum	7
Guan [39]	2021	110	Asia	SC, PC	Sepsis 3.0	Septic shock	Serum	7
Lei [40]	2022	45	Asia	SC, PC	Sepsis 3.0	Sepsis with ARDS	Serum	7
Li [41]	2018	67	Asia	SC, PC	Sepsis 3.0	Sepsis with ARDS	Serum	9
Liang [42]	2021	112	Asia	SC, RC	Not mention	Septic shock	Serum	7
Sun [43]	2022	103	Asia	SC, PC	Sepsis 3.0	Sepsis with ARDS	Serum	9
Wang [44]	2021	180	Asia	SC, PC	Sepsis 1.0	Sepsis, severe sepsis	Serum	8
Wen [45]	2021	79	Asia	SC, PC	Sepsis 3.0	Sepsis with ARDS	Serum	8
Wu [46]	2022	75	Asia	SC, PC	Sepsis 3.0	Sepsis	Serum	7
Zhang [47]	2022	85	Asia	SC, PC	Sepsis 1.0	Sepsis with ARDS	Serum	8

SC single center, *MC* multiple centers, *PC* prospective cohort study, *RC* retrospective cohort study, *SIRS* systemic inflammatory response syndrome, *ARDS a*cute respiratory distress syndrome

significant statistical heterogeneity in this subgroup (Fig. 2C).

Three studies provided 30-day mortality, six studies provided in-hospital mortality, and 16 studies provided 28-day mortality. Subgroup analysis suggested that follow-up duration was not a source of heterogeneity (30-day mortality: $I^2=73.9\%$, $P=0.022$; in-hospital mortality: $I^2=90.9\%$, $P<0.001$; 28-day mortality: $I^2=96.0\%$, $P<0.001$). The pooled results of subgroup analysis were consistent with the overall results, showing that non-survivors presented higher levels of Ang-2 than survivors (SMD=1.15, 95% CI 0.67–1.64, $P<0.001$) (Fig. 2B).

13 studies quantified plasma Ang-2 levels, while 15 studies measured serum Ang-2 levels. Subgroup analysis demonstrated that the sample sources did not contribute to the observed heterogeneity (plasma: $I^2=93.4\%$, $P<0.001$; serum: $I^2=95.6\%$, $P<0.001$). Consistent with

the overall findings, the pooled results of subgroup analyses showed that non-survivors presented higher Ang-2 levels than survivors (Supplementary Material 5).

22 studies were prospective observational studies, while six studies were retrospective. Subgroup analysis revealed that the study type was not a contributor to heterogeneity (prospective cohort study: $I^2=93.9\%$, $P<0.001$; retrospective cohort study: $I^2=96.7\%$, $P<0.001$). The pooled results of subgroup analysis were consistent with the overall results, showing that non-survivors presented higher Ang-2 levels than survivors (Supplementary Material 5).

13 studies were conducted in Asia, and 15 studies were conducted in Europe or other continents. Subgroup analysis indicated that the region was not a source of heterogeneity (Non-Asia: $I^2=90.4\%$, $P<0.001$; Asia: $I^2=94.7\%$, $P<0.001$). Consistent with the overall findings, the
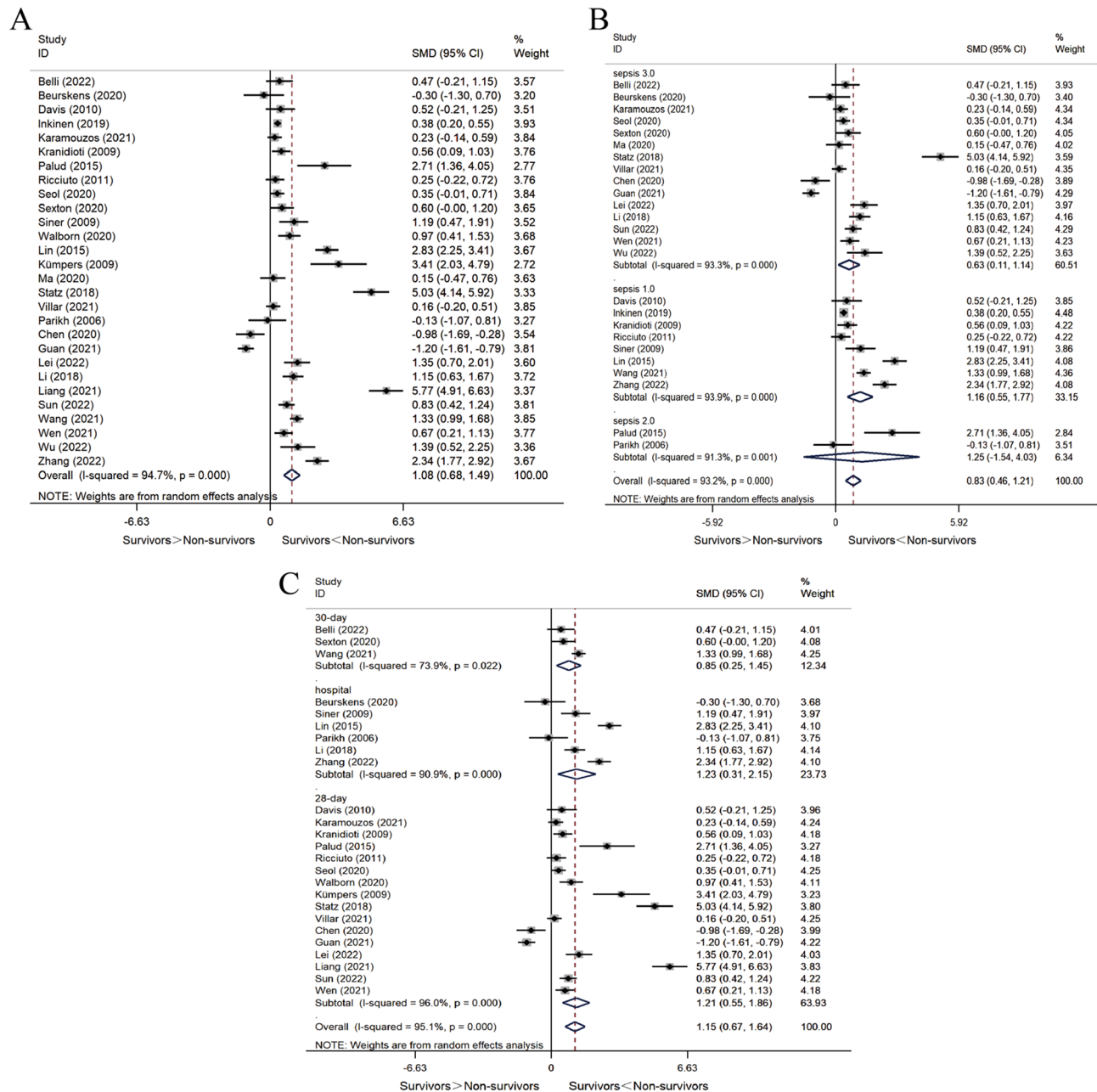
Fig. 2 Forest plots of SMD in Ang-2 biomarker measurements in survivors vs. non-survivors. When SMD exceeds 0, it implies that Ang-2 levels are higher in non-survivors compared to survivors. **A** SMD of Ang-2 levels in survivors vs. non-survivors. **B** SMD of Ang-2 levels in survivors vs. non-survivors (subgroup analysis according to follow-up duration). **C** SMD of Ang-2 levels in survivors vs. non-survivors (subgroup analysis according to sepsis diagnostic criteria)

pooled results of the subgroup analysis showed higher Ang-2 levels in non-survivors compared to survivors (Supplementary Material 5).

Sensitivity analysis confirmed the robustness of the results (Supplementary Material 6). Funnel plots and Egger's test (t=2.28, *P*=0.031) suggested publication bias. Comparison of the funnel plots before and after the trim-and-fill method revealed no new literature, maintaining

the consistency and reliability of the study results (Supplementary Material 6).

Evaluation of Ang-2 as a risk factor for mortality

We incorporated 18 studies to evaluate the association between Ang-2 and mortality in septic adult patients. The comprehensive analysis revealed a correlation between these two variables (OR=1.16, 95% CI 1.09–1.23, *P*<0.001) (Fig. 3A). These studies were divided into three
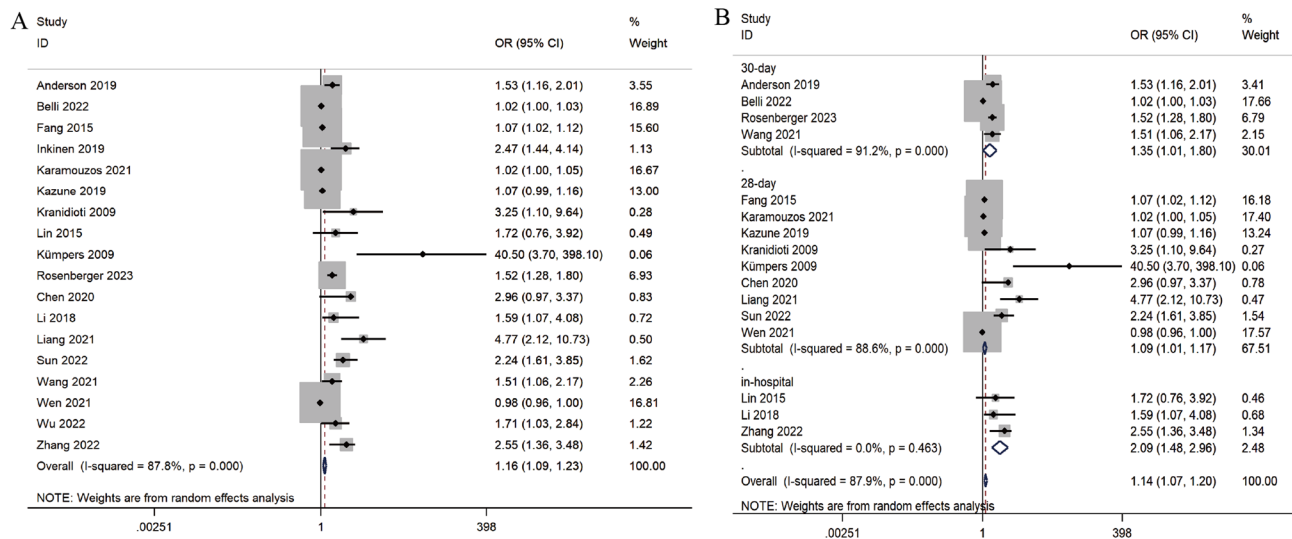
Fig. 3 Forest plots of the association between Ang-2 and mortality in septic adult patients. When OR is greater than 1, Ang-2 is a risk factor for poor outcome, otherwise, it is a protective factor. **A** Forest plot of the association between Ang-2 and mortality. **B** Forest plot of the association between Ang-2 and mortality (subgroup analysis according to follow-up duration)

subgroups based on the follow-up duration (Fig. 3B). Notably, there was considerable heterogeneity among the studies ($I^2=87.8\%$, $P<0.001$). According to the sensitivity analysis, three studies [13, 18, 42] had a notable impact on the results (Supplementary Material 6). Nevertheless, the results remained consistent after excluding these three studies, confirming the robustness of the results. Publication bias was analyzed by funnel plots and Egger's test (t=8.42, $P < 0.001$), which provided definitive evidence for publication bias. The funnel plot showed significant publication bias (Supplementary Material 6), and the number of missing studies was estimated to be eight by the trim-and-fill method (Supplementary Material 6). The funnel plot was filled to obtain the pooled effect statistic using the random-effects model (OR=1.11, 95% CI 1.04–1.18, $P<0.001$).

Estimation of the predictive performance of Ang-2 for mortality

16 of the included studies analyzed the diagnostic power of Ang-2 in predicting mortality, and 12 of these studies could be used for quantitative meta-analysis. Pooled analysis revealed an overall AUC value of 0.76 (95% CI 0.70–0.82, $P < 0.001$) (Fig. 4A). Eight studies evaluated the predictive performance of Ang-2 in predicting 28-day mortality, and the pooled analysis revealed an overall AUC value of 0.76 (95% CI 0.69–0.84, $P < 0.001$) (Fig. 4B). There was considerable heterogeneity between studies [($I^2=81.8\%$, $P<0.001$) (Fig. 4A), ($I^2=84.0\%$, $P<0.001$) (Fig. 4B)], necessitating the use of the random-effects model. Furthermore, sensitivity analysis revealed that the results were robust (Fig. 4E). According to the funnel plot and Egger's test (t = -2.68, $P=0.023$), the existence

of publication bias was confirmed. Comparison of the funnel plots before and after the trim-and-fill method revealed no new literature (Fig. 4C and D), and the recalculated overall effect statistic was 0.76 (95% CI 0.70–0.83, $P < 0.001$).

Ang/Tie-2 signaling pathway

In this meta-analysis, we included a total of 33 studies that used Ang-2 to predict mortality in septic adult patients, four of which compared Ang-2 with other molecules in the Ang/Tie-2 signaling pathway. The quantity of pertinent studies is insufficient for a comprehensive quantitative meta-analysis, and Table 2 displays the data.

Discussion

In this systematic review, we aimed to analyze the association between Ang-2 and mortality in septic adult patients, and ultimately 33 studies were included regardless of the follow-up duration. Our data showed that non-survivors exhibited higher Ang-2 levels compared to survivors. Moreover, Ang-2 was demonstrated to be able to predict mortality in septic adult patients. Therefore, Ang-2 may assist in the early identification of patients at high risk of death from sepsis.

As a member of the angiopoietins family, Ang-2 functions through the Ang/Tie-2 signaling pathway [48]. Many preclinical studies have emphasized the importance of the Ang/Tie-2 pathway in the onset and progression of sepsis [49–51]. A novel antibody, known as the ANG2-binding and TIE2-activating antibody (ABTAA), has been proven to be highly effective in protecting the endothelial barrier. It helps prevent blood components and pathogens from entering surrounding
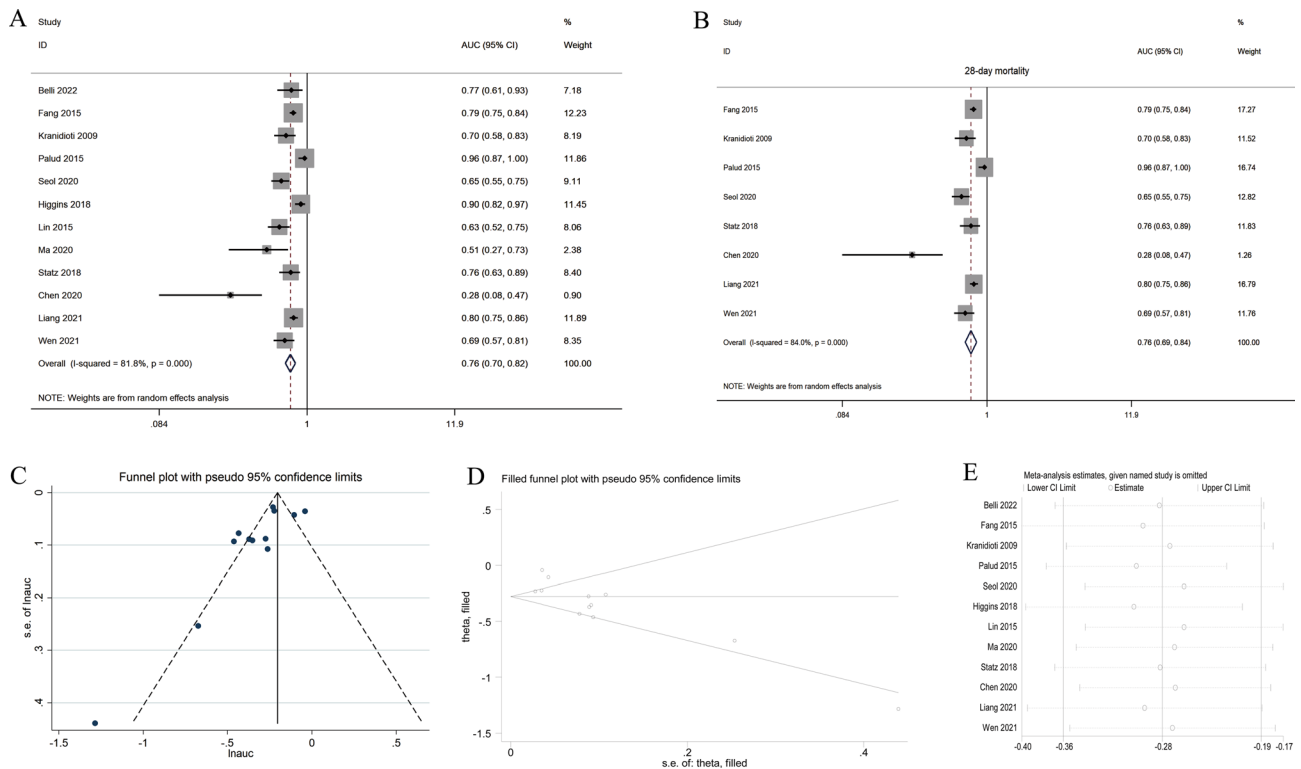
Fig. 4 Forest plots of the predictive performance of Ang-2 for mortality in septic adult patients. The closer the value of AUC is to 1, the higher the predictive performance of Ang-2 as a prognostic biomarker. **A** Forest plot of the predictive performance of Ang-2 for mortality. **B** Forest plot of the predictive performance of Ang-2 for mortality (subgroup analysis according to follow-up duration). **C** Funnel plot. **D** Filled funnel plot. **E** Sensitivity analysis

Table 2 Comparison of members of Ang/Tie-2 signaling pathway as prognostic biomarkers

	Study	Ang-2	Ang-1	Tie-2	Ang-2/Ang-1	Ang-1/Tie-2
OR (95%CI)	Belli 2022	1.02 (1.00, 1.03)	1.00 (0.99, 1.01)	-	-	-
	Fang 2015	1.07 (1.02, 1.12)	0.94 (0.80, 1.10)	1.00 (1.00, 1.07)	0.99 (0.91, 1.07)	0.01 (0.01, 0.01)
	Lin 2015	1.72 (0.76, 3.92)	2.57 (1.12, 5.90)	-	-	-
AUC (95%CI)	Fang 2015	0.79 (0.75, 0.84)	0.78 (0.73, 0,82)	0.80 (0.76, 0.85)	0.85 (0.81, 0.88)	0.81 (0.76–0.85)
	Seol 2020	0.65 (0.55, 0.75)	-	-	0.74 (0.65, 0.82)	-
	Lin 2015	0.63 (0.52, 0.75)	0.74 (0.73, 0.85)	-	-	-

tissues, protects the integrity of endothelial glycocalyx and microvasculature, alleviates vascular leakage, and reduces cytokine storms in sepsis [52]. Ang-2 has been widely studied as a key molecule in the Ang/Tie-2 pathway. Higgins et al. [30] revealed a pivotal contribution of Ang-2 to the process of sepsis, serving as the key molecule linking coagulation, inflammation, complement, and other systems. Elevated Ang-2 levels in sepsis lead to increased vascular endothelial permeability, which may aggravate severity and cause organ dysfunction and mortality [28, 49, 53]. Our meta-analysis demonstrated the prognostic significance of Ang-2 in predicting mortality

in septic adult patients. We also compared Ang-2 with other molecules in the Ang/Tie-2 pathway. However, the number of relevant studies on this comparison was insufficient for a comprehensive quantitative synthesis.

To our knowledge, Pregernig et al. [11] reported a meta-analysis evaluating Ang-2 as a biomarker for predicting mortality in adult patients with sepsis. Both our meta-analysis and the one conducted by Pregernig et al. [11] reached the consensus that Ang-2 was correlated with mortality in septic adult patients. Our meta-analysis, however, differs from that of Pregernig et al. in terms of inclusion and exclusion criteria, follow-up duration for

prognosis, types of study design, and the languages of the included studies. Of particular importance, we included sepsis patients diagnosed according to sepsis 3.0 criteria. In addition, we attempted to identify the molecule with the best prognosis-predicting performance in the Ang/Tie-2 signaling pathway.

Our study found that Ang-2 was associated with 28-day mortality, 30-day mortality, in-hospital mortality, and 90-day mortality in septic adult patients. Ang-2 predicted 28-day mortality with a reasonable degree of accuracy. We analyzed the differences in Ang-2 levels between survivors and non-survivors in the context of various sepsis diagnostic criteria. The results showed that the Ang-2 levels were higher in non-survivors in the subgroups of sepsis 1.0 and sepsis 3.0 criteria. However, there was no difference in the subgroup of sepsis 2.0 criteria. This may be related to the limited number of clinical studies due to the complexity of sepsis 2.0 criteria [54]. These results should be interpreted with caution because of the high degree of heterogeneity among the included studies. To explore the sources of heterogeneity, we conducted subgroup analysis. There are differences in genetic differences, environmental factors, and disease susceptibility in different regions, which may lead to the high heterogeneity of research results. Through subgroup analysis, we showed that regions were not the source of high heterogeneity. Villar et al. [36] compared the difference of Ang-2 levels in plasma and serum in sepsis patients requiring mechanical ventilation and found that the difference was statistically significant, which provided a reference for us to identify a second subgroup. The results of subgroup analysis indicated that specimen source (plasma or serum) was not a factor contributing to the high heterogeneity. The other two subgroups are follow-up duration and types of study. By subgroup analysis, we did not find the source of heterogeneity, but the sensitivity analysis results showed that our study results were robust and reliable. Furthermore, we sought to identify the molecule with the highest prognostic predictive efficacy in the Ang/Tie-2 signaling pathway. Although the quantities were inadequate for quantitative analysis, our summary results showed that alternative molecules or combinations of molecules did not provide substantial advantages over Ang-2 alone. Ang-2, being the most extensively studied molecule in the Ang/Tie-2 pathway, holds significant promise in predicting the unfavorable outcome of sepsis.

There are several limitations in our study. First, the included studies were all observational due to the nature of the research content. Second, due to publication bias and significant heterogeneity among the included studies, these results should be interpreted with caution. We tried to find the source of heterogeneity through sensitivity analysis and subgroup analysis, but failed. Nonetheless, the filled funnel plot and sensitivity analysis confirmed the robustness and reliability of the results to some degree. Third, we had planned to compare Ang-2 with Ang-1 or Tie-2, or a combination of them, to confirm the hypothesis, but quantitative analysis was not possible due to the lack of relevant data. Finally, some of the data was only published in Chinese.

Conclusion

Our meta-analysis suggests that Ang-2 is able to predict mortality in septic adult patients. It may facilitate the early identification of sepsis patients at high risk of poor prognosis. Additional investigations are needed to establish the optimal cut-off value prior to widespread clinical application.

Abbreviations

ABTAA	ANG2-binding and TIE2-activating antibody
AKI	Acute kidney injury
Ang-2	Angiopoietin-2
ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
CI	Confidence intervals
CMeSH	Chinese medical subject headings
HR	Hazard ratios
MC	Multiple center
MD	Mean differences
MeSH	Medical subject headings
NOS	Newcastle-Ottawa scale
OR	Odds ratios
PC	Prospective cohort study
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RC	Retrospective cohort study
SC	Single center
SIRS	Systemic inflammatory response syndrome
SMD	Standardized mean differences
SSC	Surviving Sepsis Campaign

Author contributions
MKZ and YWC conducted literature screening. XHL, MKZ and SYL carried out quality assessment and data extraction. MKZ and SFF performed data analysis. MKZ drafted the manuscript. XCM and XL revised the manuscript. XL contributed significantly to the study's conception, design, interpretation of data and overall supervision. XCM and XL obtained funding. All authors participated in the review and approval of the final manuscript.

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Data availability
All data generated and/or analyzed during the current study are included within the published article and its additional files.

Declarations

Ethical approval
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interests.

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