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Bilirubin as a predictor of severity and adverse clinical outcomes of acute ischemic stroke: a systematic review and meta-analysis

Ying Zhong[1] and Lei Li[1,2*]

Abstract

Background This review aims to comprehensively examine the role of bilirubin in predicting severity and adverse clinical outcomes in patients with acute ischemic stroke (AIS).

Methods We searched the electronic PubMed, Embase, Scopus, and Web of Science repositories for articles published in English available before the 15th of June 2024. The outcomes assessed were stroke severity, haemorrhagic transformation, symptomatic intracranial haemorrhage (sICH), mortality, and poor functional results.

Results We analysed data from 13 studies. Our meta-analysis showed that both total bilirubin (RR, 1.10; 95% CI, 1.01–1.19) and direct bilirubin (RR, 1.79; 95% CI, 1.33–2.42) were independently associated with the severity of AIS. Higher quartiles of total bilirubin were associated with an increased risk of haemorrhagic transformation, but without statistical significance (RR, 2.34; 95% CI, 0.90–6.07). In addition, each unit increase in direct (RR, 1.25; 95% CI, 1.09–1.43) and indirect (RR, 1.09; 95% CI, 1.02–1.17) bilirubin levels was significantly associated with a higher risk of haemorrhagic transformation. Moreover, each unit increases in total (RR, 1.08; 95% CI, 1.04–1.12), direct (RR, 1.28; 95% CI, 1.13–1.44), and indirect (RR, 1.10; 95% CI, 1.03–1.18) bilirubin levels was significantly associated with a higher risk of sICH. Data on mortality and poor functional outcomes were insufficient.

Conclusion Serum bilirubin levels were positively associated with the severity of AIS. The evidence suggests that bilirubin may be a potential indicator for haemorrhagic transformation and sICH after AIS.

Keywords Stroke, Cerebrovascular accident, Liver function, Haemorrhagic transformation

Introduction

Acute ischemic stroke (AIS) is defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction [1]. Stroke is the 2nd leading cause of death worldwide causing approximately 1/3rd of all disabilities [2]. In 2016, there were more than 13 million new cases of stroke [3]. Chinese data from 2020 showed that approximately 3.4 million patients experienced a first-ever stroke resulting in 2.3 million deaths [4]. Despite advances in therapeutic interventions, access to these modalities for AIS remains limited [5, 6]. Globally, less than 5% of patients with AIS receive intravenous

*Correspondence:
Lei Li
chenjinshijin1@163.com
[1]Geriatrics Department, Huzhou Third Municipal Hospital, the Affiliated Hospital of Huzhou University, Huzhou, Zhejiang, China
[2]Geriatrics Department, Huzhou Third Municipal Hospital, the Affiliated Hospital of Huzhou University, No. 2088 East Tiaoxi Road, Huzhou, Zhejiang Province, China

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thrombolysis within the eligible therapeutic time window [3]. The 30-day mortality after AIS is approximately 11%, and every two of three patients die or are left with dependent disabilities after 5 years [7]. Likewise, a large proportion of patients with AIS are left with long-term functional problems after a stroke [1].

Stroke severity is often assessed via validated scores like the National Institutes of Health Stroke Scale (NIHSS) which also provide an indication of prognosis. However, even the NIHSS has limitations [8], and other biomarkers supplementing such validated tools are needed to improve the accuracy of stroke prognoses and severity assessments. Indeed, prognostic factors that can help prioritize patients with stroke at risk for worse survival or dependence have been identified like the brain natriuretic peptide, D-dimer, matrix metallopeptidase-9, S100B, C-reactive protein, and nutritional markers like geriatric nutritional risk index, prognostic nutritional index, and controlling nutritional status score [9]. However, no single marker has gained global acceptability as many markers are not widely accessible in clinical practice and results have varied between populations [10].

Bilirubin is a by-product of heme metabolism which indirectly represents the functioning of the liver and is a widely used marker for detecting diseases of the hepatobiliary system. However, bilirubin is also a potent physiological antioxidant [11] used as a marker for various diseases. Serum bilirubin levels have been associated with Parkinson's disease [12], diabetes mellitus complications [13], and chronic kidney disease progression [14]. Further, several cardiovascular ailments share an association with bilirubin levels. There is an inverse association between bilirubin levels and the severity of coronary artery disease (CAD) [15]. Serum bilirubin is also a prognostic marker for pulmonary hypertension and heart failure [16, 17]. Similarly, studies have reported an association between bilirubin levels and AIS. A large meta-analysis of 131,450 individuals showed that elevated bilirubin levels reduce the risk of AIS [18]. Another meta-analysis showed that bilirubin levels are lower in patients with AIS than in healthy controls, but higher bilirubin levels may lead to increased stroke severity [19]. Given the association between bilirubin levels and the incidence of stroke and the studies reporting bilirubin as a prognostic indicator for other cardiovascular diseases [16, 17], clarifying the association between bilirubin and clinical outcomes after AIS is important. To the best of our knowledge, this is the first systematic review thoroughly examining the role of serum bilirubin in predicting the severity and clinical outcomes of AIS.

Materials and methods

We prospectively registered this PRISMA [20] guideline-based review on the international PROSPERO database (https://www.crd.york.ac.uk/prospero/) with registrati on number CRD42024555898. No ethical approval was required as this review included already published data.

Inclusion criteria

We established inclusion criteria prior to beginning the literature search. We included all types of studies conducted on AIS patients reporting AIS outcomes and baseline bilirubin levels (reported as total bilirubin [TB], direct bilirubin [DB], or indirect bilirubin [IB]). Outcomes of interest were AIS severity, haemorrhagic transformation, symptomatic intracranial haemorrhage (sICH), mortality, and poor functional outcomes. We accepted all the different outcome definitions used by the studies. Similarly, no cut-off for high bilirubin was predefined, and we used the values determined by the studies. For inclusion, studies had to report multivariable adjusted data of any of the above outcomes.

We excluded studies that failed to segregate outcomes based on bilirubin levels in addition to studies not assessing outcomes by multivariate analysis, reporting numerical data, or with duplicate data. Whenever the sample size overlapped between two articles, we chose the study with the maximum sample size or reporting the most outcomes. The corresponding authors were not contacted for any missing data. Lastly, we did not include data from abstracts, reviews, or unpublished data in our analysis.

Search protocol

Two reviewers (YZ, LL) searched the electronic repositories of PubMed, Embase, Scopus, and Web of Science for articles published in English and available before 15th June 2024. The medical librarian of our hospital helped the reviewers with the literature search. The search was carried out using full-text keywords with the following query: ((((stroke) OR (cerebral infarction)) OR (cerebral accident)) OR (cerebrovascular accident)) AND ((bilirubin) OR (Hematoidin)). The query was replicated across all databases. The bibliography of eligible articles was also scanned to identify any missed studies. We also searched Google Scholar to include gray literature.

All search results were combined in a single reference manager software (EndNote). Duplicates were eliminated. Two authors (YZ, LL) separately read the titles/abstracts of the studies and identified potentially relevant studies. During the second phase, the two authors (YZ, LL) examined the full texts and removed reports that did not fulfil the eligibility criteria. Disagreements were resolved by discussion.

Data management

Two reviewers (YZ, LL) extracted the data extraction collecting information on a standardized form which included the author's name, year of publication, location, study design, sample size, demographic details, comorbidities, type of bilirubin measured, cut-off, percentage with highest bilirubin values, factors adjusted in the multivariate analysis, follow-up information, and outcomes.

We tabulated all outcome data and separated bilirubin data as a categorical continuous variable. Data were also segregated for TB, DB, and IB. We conducted a meta-analysis whenever the exposure and data analysis were similar in at least two studies. We conducted a qualitative analysis whenever a meta-analysis was not feasible.

Risk of bias

Given the observation nature of the study, we chose to use the Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E) tool to assess study quality [21]. The tool contains predefined criteria covering the following domains: bias due to confounding, bias arising from the measurement of the exposure, bias in the selection of participants into the study, bias due to post-exposure interventions, bias due to missing data, bias arising from the measurement of the outcome, and bias in the selection of the reported result. Two reviewers (YZ, LL) conducted the quality assessment and resolved all disagreements by discussion.

Statistical analysis

The statistical analysis including group combining was done in "Review Manager" (RevMan, version 5.3; Nordic Cochrane Centre [Cochrane Collaboration], Copenhagen, Denmark; 2014). We pooled adjusted data to obtain risk ratios (RRs) and 95% confidence intervals (CIs) for all outcomes. The effect estimates from the studies were combined using a random-effects model. The I^2 statistic estimated the proportion of variation across studies attributable to heterogeneity rather than chance [22]. Values of $I^2 > 50\%$ indicated substantial heterogeneity. Given the limited number of studies available for the meta-analysis, we did not generate funnel plots to examine publication bias. A leave-one-out analysis was conducted to identify any outlier studies in the meta-analysis. This was done when there were at least three studies in the meta-analysis to provide meaningful results. We also assessed the certainty of evidence using GRADE [22].

Results

Study selection

We found 1336 articles from all databases; 752 studies were duplicates and hence removed. The remaining 584 articles were screened and 25 were selected by the reviewers for full-text review. Inter-reviewer agreement was high (*kappa* = 0.9). We finally selected 13 studies [23–35] for this review (Fig. 1). The bibliography and grey literature search revealed no additional studies.

Study characteristics

Table 1 shows all baseline study information. Except for two studies from the USA, all other studies were on Chinese populations. The publication years ranged between 2008 and 2023. Three articles were from retrospective cohort studies, all others were cross-sectional studies. We analysed data from 20,198 patients with AIS. Four studies reported only TB, whereas two studies used only DB as the exposure variable. The remaining studies reported multiple bilirubin values. There was much variation in the cut-off for high TB, DB, or IB in the sample. Similarly, the adjusted factors for the multivariate analysis also differed across studies. The risk of bias based on the ROBIN-E tool is presented as Fig. 2. All studies had a high to very high risk of bias except for one study which had a low risk of bias. Table 2 presents the study outcomes, definitions, effect size, and study conclusions.

Stroke severity

The association between bilirubin and AIS severity was reported by five studies, all of which found a positive correlation between bilirubin levels and AIS severity. All the studies used the National Institutes of Health Stroke Scale to define stroke severity but used different cut-offs. Meta-analysis results showed that TB (RR, 1.10; 95% CI, 1.01–1.19 $I^2 = 88\%$ $p = 0.03$) and DB (RR, 1.79; 95% CI, 1.33–2.42 $I^2 = 89\%$ $p = 0.0001$) had a statistically significant association with AIS severity (Fig. 3). High heterogeneity was noted in both the meta-analyses. The significance of the results did not change during the leave-one-out analysis. Duan et al. [23] applied a multivariable linear regression analysis instead of a multivariable logistic regression and hence could not be included in our meta-analysis.

Haemorrhagic transformation

Four studies reported on haemorrhagic transformation. Bilirubin levels were associated with a high risk of hemorrhagic transformation in all studies. But there were differences in the presentation of data with some using bilirubin as categorical and others using it as a continuous variable. Pooling similar data together we noted that higher quartiles of TB were not significantly associated with an increased risk of hemorrhagic transformation (RR, 2.34; 95% CI, 0.90–6.07, $I^2 = 90\%$ $p = 0.08$). Exclusion of the study of Tan et al., however, showed a significant association between TB and hemorrhagic transformation (RR, 3.70; 95% CI, 2.22–6.18, $I^2 = 0\%$ $p < 0.0001$). Meta-analysis of just two studies each showed that per unit increase of DB (RR, 1.25; 95% CI, 1.09–1.43, $I^2 = 40\%$

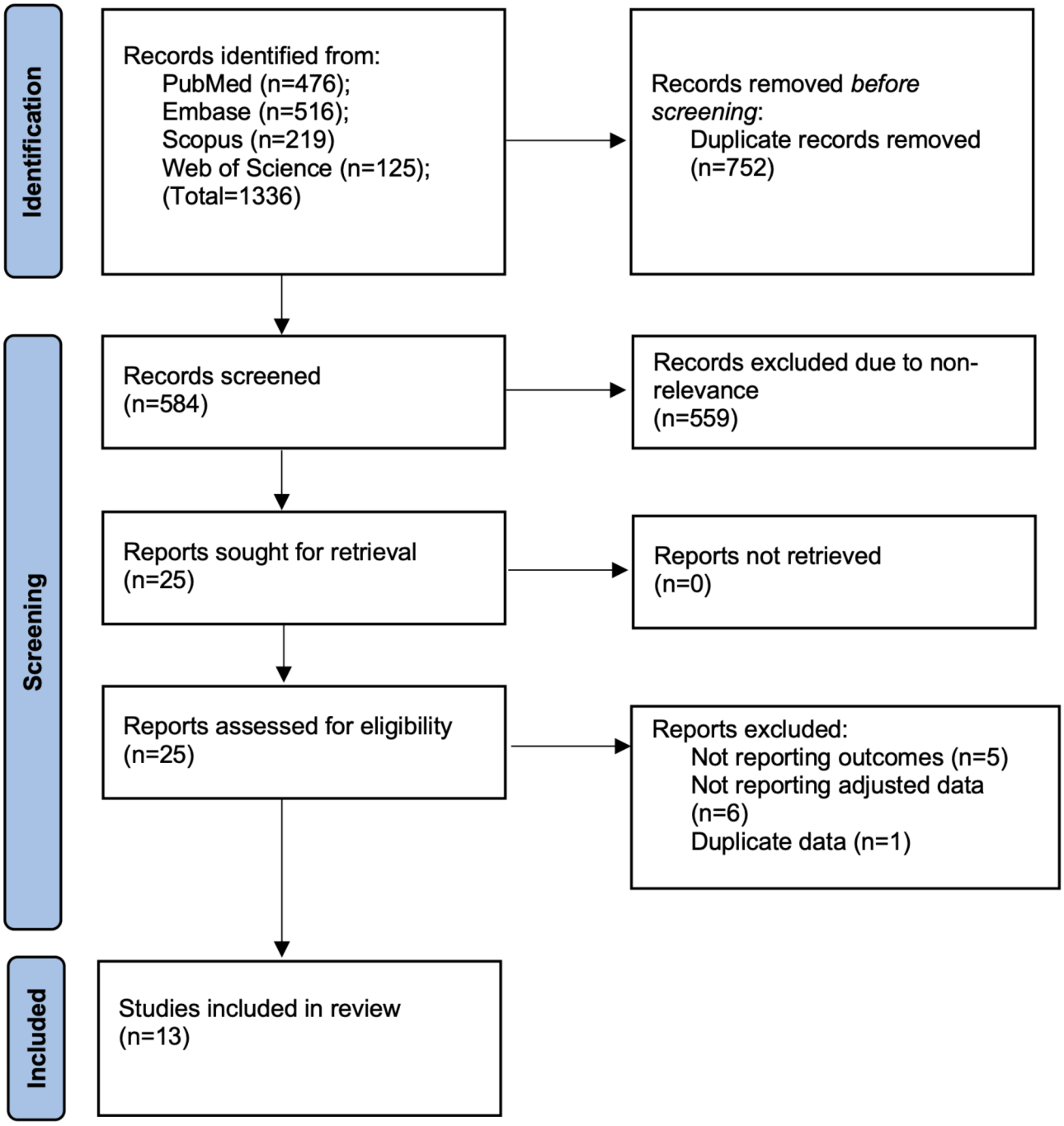

Fig. 1 PRISMA chart depicting search results

$p = 0.02$) and IB (RR, 1.09; 95% CI, 1.02–1.17, $I^2 = 62\%$ $p = 0.02$) were significantly associated with a higher risk of haemorrhagic transformation (Fig. 4).

sICH

Three studies reported sICH data. Of these, two found a significant association between bilirubin and sICH. All three studies reported the risk of sICH with bilirubin as a continuous variable. Our meta-analysis results showed that each unit increase in TB (RR, 1.08; 95% CI, 1.04–1.12, $I^2 = 0\%$ $p < 0.0001$), DB (RR, 1.28; 95% CI, 1.13–1.44, $I^2 = 0\%$ $p < 0.0001$), or IB (RR, 1.10; 95% CI, 1.03–1.18, $I^2 = 29\%$ $p = 0.004$) was significantly associated with a higher risk of sICH (Fig. 5). These results did not change in significance during the leave-one-out analysis. When examined as a categorical variable, a statistically significant increased sICH risk was identified only with the higher DB levels (RR, 3.88; 95% CI, 1.44–10.45, $I^2 = 28\%$

Table 1 Baseline details of included studies

Study	Location	Design	Sample size	Males (%)	Age (years)	DM (%)	HT (%)	DL (%)	AF (%)	Exp-osure	Exposure level (umol/L)	% with high level	Adjusted factors	FU
Perlstein 2008 [26]	USA	CS	453	NR	NR	NR	NR	NR	NR	TB	Highest: 0.8–12.9 Lowest: 0.1–0.5	26.9	Age, sex, race/ethnicity, smoking, hypertension, total to HDL cholesterol ratio, and diabetes	NR
Pineda 2008 [25]	USA	CS	743	47.6	67.5	24.3	67	NR	19.7	DB	Highest: ≥0.4 Lowest: ≤0.1 (mg/dl)	5	Sex, serum glucose, prior antithrombotic use, hypertension, atrial fibrillation, and mRS scores	NR
Luo 2012 [24]	China	CS	531	63.5	67	NR	63.4	NR	14	TB DB	Highest: TB ≥ 22.2; DB ≥ 6.84 Lowest: TB ≥ 10.2; DB ≤ 3.42	TB: 25.4 DB: 18	Age, sex, hypertension, atrial fibrillation, HDL, cholesterol	NR
Xu 2013 [34]	China	CS	2361	63.2	63.9	13.4	61.4	38.1	3.1	TB DB	Highest: TB 18–88; DB 4.2–37 Lowest: TB 1–10; DB 0.4–2.0	NR	Age, sex, alcohol consumption, cigarette smoking, blood levels of glucose and lipids, blood pressure, blood urea nitrogen, serum creatinine, sodium, hematocrit, history of stroke, hypertension, diabetes, coronary heart disease, rheumatic heart disease, and atrial fibrillation, family history of stroke, hypertension, and diabetes	NR
Tan 2015 [33]	China	CS	2788	60.1	63.5	27.1	62.2	20	19.4	TB	Highest: ≥18 Lowest: <9.8	25.5	DL, AF, NIHSS, glucose, AST	NR
Geng 2016 [32]	China	CS	301	57.1	64.1	19.3	60.8	NR	NR	DB	Continuous variable	NR	Age, residence, recurrent ischemic stroke, coronary heart disease, NIHSS score at admission, lacunar stroke, time from onset of stroke to admission, C reactive protein, albumin, fibrinogen and D-dimer	Discharge
Jian 2020 [31]	China	CS	153	61.4	65.4	42.5	57.5	33.3	40.5	TB, DB, IB	Continuous variable	NR	Age, NIHSS, coronary artery disease, glucose, cardioembolic stroke	48 h
Li 2020 [28]	China	CS	610	63.1	66.7	33.1	73.1	NR	NR	TB, DB	NR	NR	HDL, cholesterol, triglyceride	NR
Ouyang 2021 [30]	China	RC	10,339	NR	NR	NR	NR	NR	NR	TB, DB, IB	Highest: TB ≥ 17.67 Lowest: TB ≤ 9.9	NR	Age, sex, history of diabetes, AF, smoking status, stroke subtype, TOAST, hypoglycemic agents, antiplatelet agents, anticoagulant agents, baseline NIHSS, total cholesterol, HDL cholesterol, triglyceride, high sensitivity C-reactive protein, alanine aminotransferase and AST	1 year
Peng 2021 [29]	China	RC	588	66.5	64.9	28	64.6	21	NR	TB, DB, IB	Highest: TB ≥ 14.2; DB ≥ 5.1, IB ≥ 9.5 Lowest: TB < 7.8; DB < 2.7; IB < 4.8	25.2 (All)	Age, sex, onset-time to treatment, admission glucose level, alanine aminotransferase, AST, smoking, alcohol drinking, history of stroke, cerebral hemorrhage, HT, DM, DL, admission NIHSS score	3 months
Chen X 2023 [27]	China	CS	557	66.5	70	27.4	77.7	31.4	27.4	TB, DB, IB	Highest: TB ≥ 20.6; DB ≥ 5.3, IB ≥ 15.6 Lowest: TB ≤ 11.8; DB ≤ 2.7; IB ≤ 8.8	NR	age, sex, NIHSS score, endovascular treatment, stroke etiology, international normalized ratio, and uric acid	36 h

Table 1 (continued)

Study	Location	Design	Sample size	Males (%)	Age (years)	DM (%)	HT (%)	DL (%)	AF (%)	Exp-osure	Exposure level (umol/L)	% with high level	Adjusted factors	FU
Chen J 2023 [23]	China	RC	247	71.3	67	26.3	61.1	NR	13.4	TB	Highest: ≥21 Lowest: <9	48.6	Age, sex, AF, HT, DM, baseline mRS, NIHSS score, white blood cell count, glucose level, platelet count, gamma-glutamyl transpeptidase, level, anticoagulant and anti-platelet therapies, hemoglobin, glucose, AST	7 days
Duan 2023 [22]	China	CS	527	69.8	64	28.1	68.5	13.1	11.4	TB	> 20	20.5	Sex, age, HT, AF, AST, lipid profile, arterial embolectomy &/or emergency stent implantation, Intravenous thrombolysis	NR

DM, diabetes mellitus; HT, hypertension; DL, dyslipidemia; AF, atrial fibrillation; NR, not reported; TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin; HDL, high density lipoprotein; mRS, modified ranking scale; NIHSS, National Institutes of Health Stroke Scale; CS, cross-sectional; RC, retrospective cohort; AST, aspartate aminotransferase

$p = 0.007$) but not with TB (RR, 2.20; 95% CI, 0.51–9.47; $I^2 = 68\%$ $p = 0.29$) or IB (RR, 1.68; 95% CI, 0.47–6.01, $I^2 = 66\%$ $p = 0.42$) levels (Fig. 6).

Functional outcomes

Three studies reported the association between bilirubin and functional outcomes after AIS. Two studies used the modified Rankin Scale (mRS) to define poor functional outcomes with the same cut-off (> 2). However, one of them used bilirubin as a continuous variable, whereas the other used it as a categorical variable. Perlstein et al. [27] reported adverse functional outcomes after stroke but defined them as either physical, mental, or emotional. Given the data heterogeneity, we did not conduct a meta-analysis. In terms of study results, both studies using the mRS found a positive association between higher bilirubin levels and poor functional outcomes, whereas Perlstein et al. [27] found that a higher serum TB level was associated with better functional outcomes.

Mortality

Data on mortality was scarce with only one study reporting outcomes. Peng et al. [30] found that DB levels (but not TB or IB levels) were independently associated with a higher risk of mortality after AIS.

Certainty of evidence

GRADE assessment of evidence is shown in Supplementary Table 1. All outcomes in the meta-analysis had "very low" certainty of evidence.

Discussion

A comprehensive review of the literature yielded 13 studies examining the association between bilirubin levels and severity or clinical outcomes of AIS. Our findings after the meta-analysis found a positive association between bilirubin levels and stroke severity. Also, higher bilirubin levels are significantly correlated with an increased risk of haemorrhagic transformation and sICH. Data on functional outcomes and mortality were either insufficient or conflicting to derive strong conclusions.

The role of bilirubin as a risk and prognostic indicator has attracted attention for different cardiovascular disorders. In a cohort study of 299 patients with CAD, Turfan et al. [36] found that lower TB levels were associated with higher syntax scores indicating a more severe disease. A different study categorizing 1501 patients with CAD based on Gensini scores also found higher TB levels in the control group than in those with severe CAD [15]. A study on patients with diabetes mellitus found that TB levels correlated negatively with lower limb plaques and stenosis [37]. This association was persistent when the TB levels were measured as a continuous or categorical variable. In a cross-sectional study of > 8000 patients, Jin

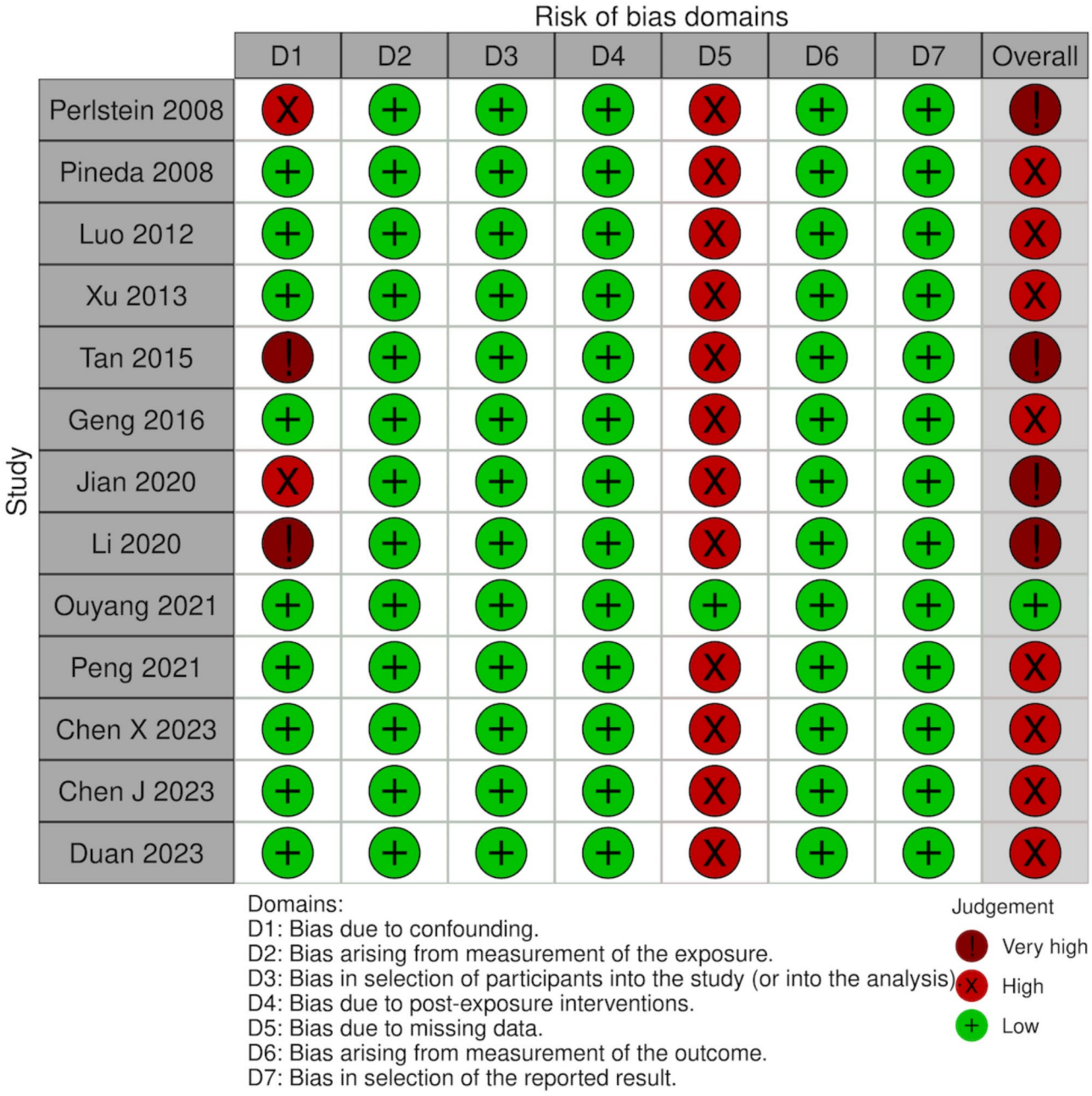

Fig. 2 Risk of bias analysis

et al. [38] showed that IB levels are inversely related to the risk of carotid plaques and stenosis, with higher IB levels being protective of carotid atherosclerosis. Based on the evidence, there seems to be an inverse relationship between bilirubin levels and the risk and severity of cardiovascular diseases. However, a similar but positive association has been observed between bilirubin levels and clinical outcomes of cardiovascular disorders. Yin et al. [39] have shown that TB levels are independent predictors of major adverse cardiac events in patients with myocardial infarction and non-obstructive coronary arteries. Similarly, higher TB levels have been associated with a 60% and 81% increase in the risk of major adverse cardiac events and cardiovascular death, respectively, in patients undergoing percutaneous coronary interventions [40]. Moreover, a published meta-analysis found that higher TB levels are associated with a higher risk of adverse outcomes in patients with myocardial infarction [41].

Our current systematic review yielded consistent findings with those in the literature in terms of AIS outcomes. Additionally, we have built upon the previous

Table 2 Details of outcomes reported by the studies

Study	Outcomes	Definition	Effect size with 95% confidence intervals	Study conclusions
Perlstein 2008 [26]	Adverse AIS outcome	Long- term physical, mental, or emotional problem or illness as a consequence of AIS	0.55 (0.31–0.98)	Higher serum TB is associated improved AIS outcomes
Pineda 2008[25]	AIS severity	NIHSS > 12	2.785 (1.25–6.202)	Higher DB is associated with greater AIS severity
Luo 2012 [24]	AIS severity	NIHSS ≥ 8	TB: 3.55 (1.57–7.15) DB: 3.70 (1.98–6.92)	Serum bilirubin's were in significant correlation with severity of AIS
Xu 2013[34]	AIS severity	NIHSS ≥ 10	TB: 2.34 (1.58–3.46) DB: 3.08 (2.05–4.62)	Serum bilirubin levels closely correlate with AIS severity
Tan 2015 [33]	HmT	Hemorrhage within the acute ischemic lesion detected by magnetic resonance imaging	1.139 (1.008–1.286)	TB independently correlated with HmT in AIS
Geng 2016 [32]	Poor functional outcome	mRS > 2	1.795 (1.311–2.458)	DB is significantly associated with discharge outcome in AIS
Jian 2020 [31]	HmT, sICH	HmT was diagnosed and classified according to the European Cooperative Acute Stroke Study radiological classification. sICH defined based on Heidelberg Bleeding Classification;	sICH TB: 1.102 (1.027–1.182) DB: 1.192 (0.967–1.471) IB: 1.177 (1.064–1.303) HmT TB: 1.106 (1.041–1.175) DB: 1.364 (1.133–1.641) IB: 1.143 (1.052–1.242)	Elevated admission bilirubin is an independent predictor of HmT and sICH in AIS patients
Li 2020 [28]	AIS severity	NIHSS ≥ 8	TB: 1.05 (1.03–1.07); 1.05 (1.02–1.09) DB: 1.18 (1.08–1.29); 1.18 (1.05–1.33)	Bilirubin independently mediates severity of AIS
Ouyang 2021 [30]	Poor functional outcome	mRS > 2	TB: 1.31 (1.08–1.58) DB: 1.43 (1.15–1.77) IB: 1.28 (1.05–1.55)	Elevated levels of serum bilirubin were significantly associated with poor functional outcomes in patients with AIS

Table 2 (continued)

Study	Outcomes	Definition	Effect size with 95% confidence intervals	Study conclusions
Peng 2021 [29]	Mortality, sICH	sICH was diagnosed as any hemorrhagic transformation temporarily associated with deterioration of neurological symptoms using the National Institute of Neurological Disorders and Stroke criteria	sICH As different groups- TB: 1.119 (0.421–2.972) DB: 2.549 (0.897–7.242) IB: 0.906 (0.343–2.398) Per unit increase: TB: 1.177 (0.861–1.610) DB: 1.555 (1.057–2.287) IB: 1.116 (0.816–1.527) Mortality As different groups- TB: 1.927 (0.758–4.899) DB: 5.872 (1.671–20.640) IB: 1.524 (0.617–3.763) Per unit increase: TB: 1.246 (0.919–1.689) DB: 1.555 1.557 (1.090–2.224) IB: 1.217 (0.906–1.635)	Increased DB pre-thrombolysis had a stronger association with as well as greater incremental predictive value for poor outcomes than TB and IB in AIS patients
Chen X 2023 [27]	HmT, sICH	HmT was diagnosed as new hemorrhage in follow-up computed tomography images within 24–36 h after thrombolysis. sICH was defined as HmT accompanied by deterioration of neurological function	HmT As different groups- TB: 3.36 (1.46–7.72) DB: 4.18 (1.74–10.00) IB: 2.37 (1.12–5.03) Per unit increase- TB: 1.05 (1.01–1.08) DB: 1.18 (1.05–1.31) IB: 1.06 (1.02–1.10) sICH As different groups- TB: 4.99 (1.32–18.94) DB: 7.13 (1.84–27.73) IB: 3.33 (1.08–10.25) Per unit increase- TB: 1.07 (1.02–1.13) DB: 1.29 (1.10–1.50) IB: 1.07 (1.02–1.13)	A positively linearly relationship is noted between serum bilirubin levels and the risk of HmT and sICH in patients with AIS undergoing intravenous thrombolysis

Table 2 (continued)

Study	Outcomes	Definition	Effect size with 95% confidence intervals	Study conclusions
Chen J 2023 [23]	HmT	HmT was diagnosed as new hemorrhage in follow-up computed tomography images	3.924 (2.051–7.505)	TB is associated with a high risk of HmT in AIS
Duan 2023 [22]	AIS severity	NIHS > 5	0.107 (0.053–0.162)*	High TB and DB level within 48 h of symptom onset could be an independent marker of severity of AIS

AIS, acute ischemic stroke; sICH, symptomatic intracranial hemorrhage; HmT, hemorrhagic transformation; NIHSS, National Institutes of Health Stroke Scale; mRS, modified ranking scale; TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin

*used multivariate linear regression analysis

meta-analyses [18, 19] supporting the use of bilirubin as a predictor of AIS severity and prognosis. Our pooled analysis found that increasing TB and DB levels were associated with more severe AIS. Although these results are consistent with those in a prior meta-analysis [19], they differ from the results on CAD [15]. Importantly, our systematic review showed that all of the included studies consistently reported a positive association, a finding that increases the credibility of these results. Differences with other cardiovascular diseases could be attributable to dissimilar study populations and pathophysiologies of the different illnesses, which deserve further investigations.

We further analysed the influence of bilirubin levels on four important outcomes namely, haemorrhagic transformation, sICH, functional outcomes, and mortality. Even after including 13 studies and conducting a comprehensive literature search, the association between bilirubin and AIS outcomes remains unclear. Inconsistent outcome reporting, variations in the type of bilirubin studied, and differences in data management are important barriers to the interpretation of the current evidence. Therefore, we chose to present a thorough systematic review of outcomes followed by a meta-analysis of similar data to present the best possible evidence. Our review revealed that bilirubin may be a potential predictor of haemorrhagic transformation and sICH in AIS. Increasing levels of TB, DB, and IB were associated with a higher risk of haemorrhagic transformation and sICH in most studies. This result was replicated in the pooled analysis, but with somewhat inconsistent results when bilirubin was a categorical variable. The risk of haemorrhagic transformation was increased but non-significant with TB levels. Similarly, when bilirubin was a categorical variable in the meta-analysis of sICH, we found a significant association only for DB levels but not for those of TB or IB. One possible reason for such inconsistencies is the variability in bilirubin cut-offs used by the included studies. All cut-offs were predetermined by each study and were specific to each cohort. Most studies reported high TB levels as >17 μmol/L, but some like the one by Perlstein et al. [27] defined high TB levels as those between 0.8 and 12.9 μmol/L. We excluded this study from the meta-analysis. The low cut-off in that study could be the reason for the better outcomes observed with high TB levels; other studies observed poor mRS scores in the higher TB group. Given the insufficiency of the data, further studies are warranted.

The pathophysiological association between bilirubin and stroke is complex and reflects the fact that bilirubin is associated with a reduced risk of stroke, stroke severity and poor clinical outcomes. The evidence indicates that bilirubin has antioxidant, anti-inflammatory, and cytoprotective properties vital for myocardial and neural tissues which lack inherent cytoprotective defence

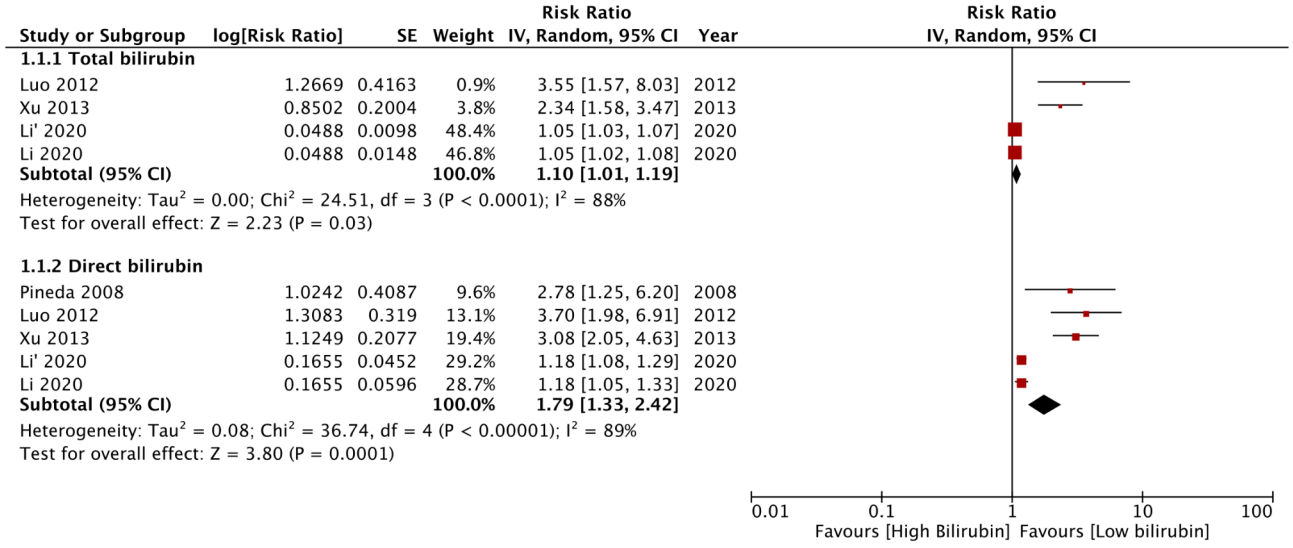

Fig. 3 Meta-analysis of AIS severity based on serum bilirubin levels

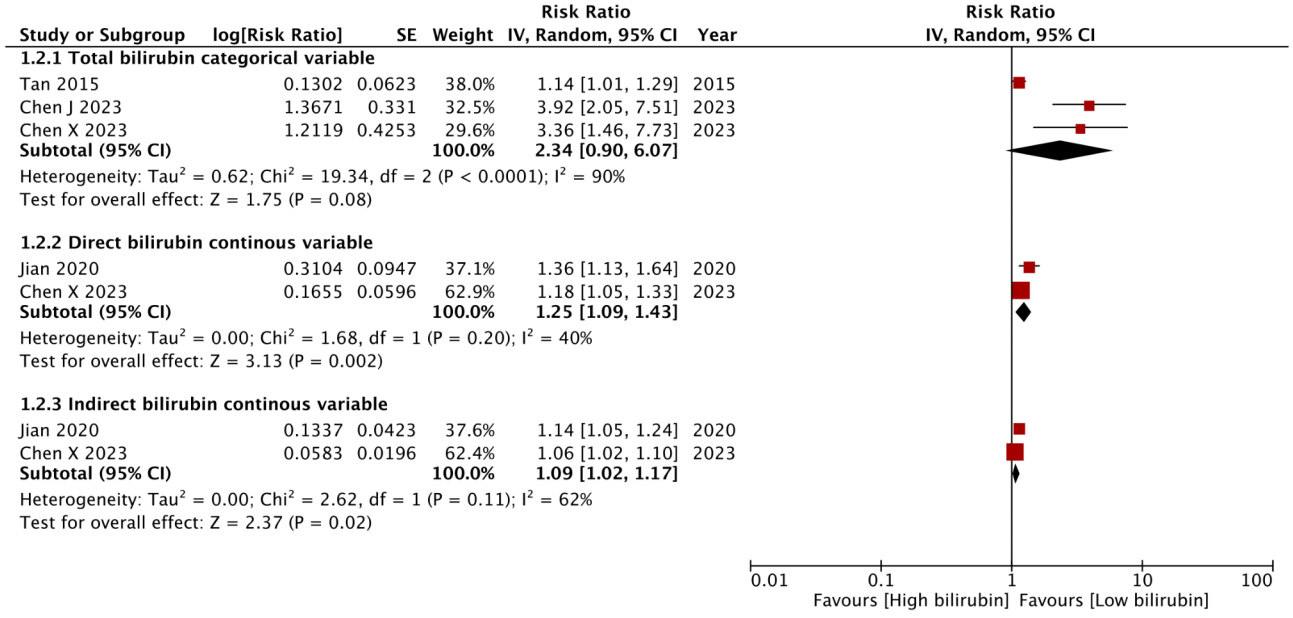

Fig. 4 Meta-analysis of haemorrhagic transformation of AIS based on serum bilirubin levels

mechanisms [42]. AIS is characterized by a sudden loss of blood supply depleting the oxygen and glucose supply to the brain. An inflammatory response ensues with overproduction of chemokines, cytokines, and reactive oxygen species which can lead to structural brain damage [19]. Superoxide and reactive oxygen species increase lipid peroxidation and the expression of proinflammatory cells in blood vessels, causing endothelial dysfunction and atherogenesis progression. Excessive ROS generation can also lead to functional and structural damage of brain cells during the whole course of stroke, especially the early phase, thereby increasing the brain insult. Bilirubin acts as a strong endogenous antioxidant by its extended conjugated double-bond system and a reactive hydrogen atom. It rapidly scavenges free radicals, preventing the deleterious effects of ischemic reactions [42]. Therefore, by its antioxidant action, it may decrease the severity of stroke. In addition, bilirubin alters the endothelial environment by reducing the expression of adhesion molecules, thereby inhibiting adherence and migration of inflammatory cells to the vessel wall. This inhibits the process of atherogenesis [43]. By contrast, the same antioxidant properties of bilirubin can become toxic. In vitro experimental results showed that bilirubin has a time-dependent variable effect on the integrity of human brain microvascular endothelial cells: Initial exposure to IB

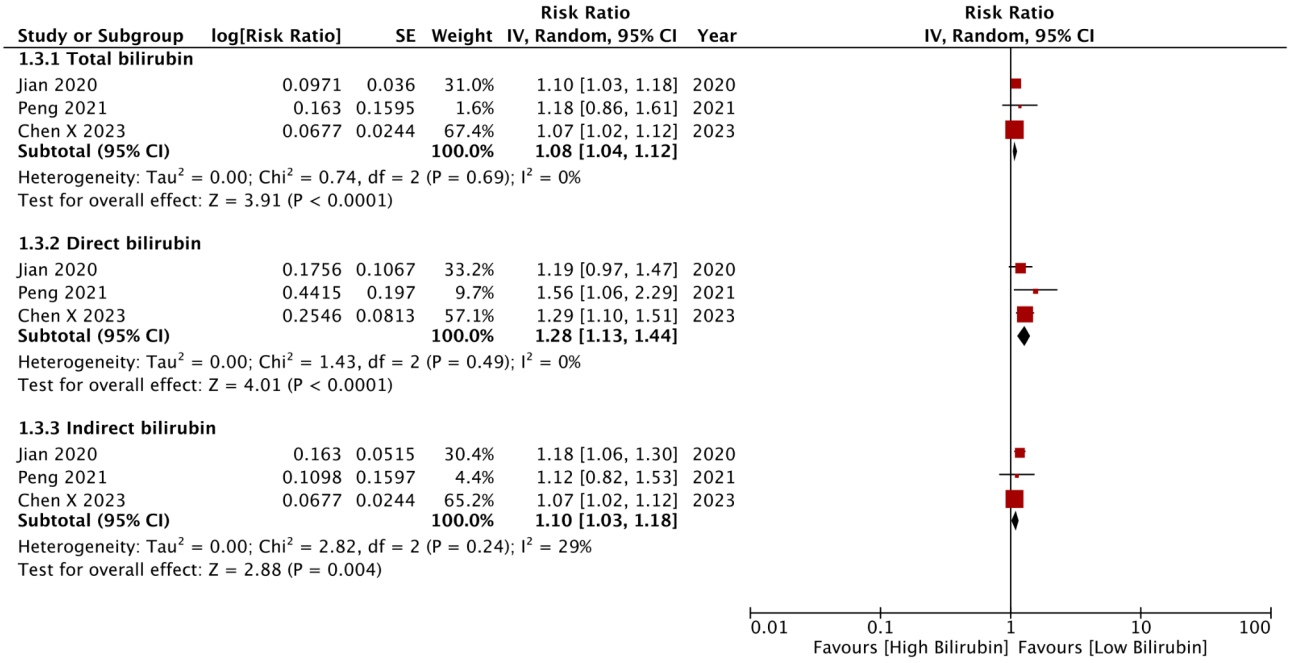

Fig. 5 Meta-analysis of sICH after AIS based on serum bilirubin levels as a continuous variable

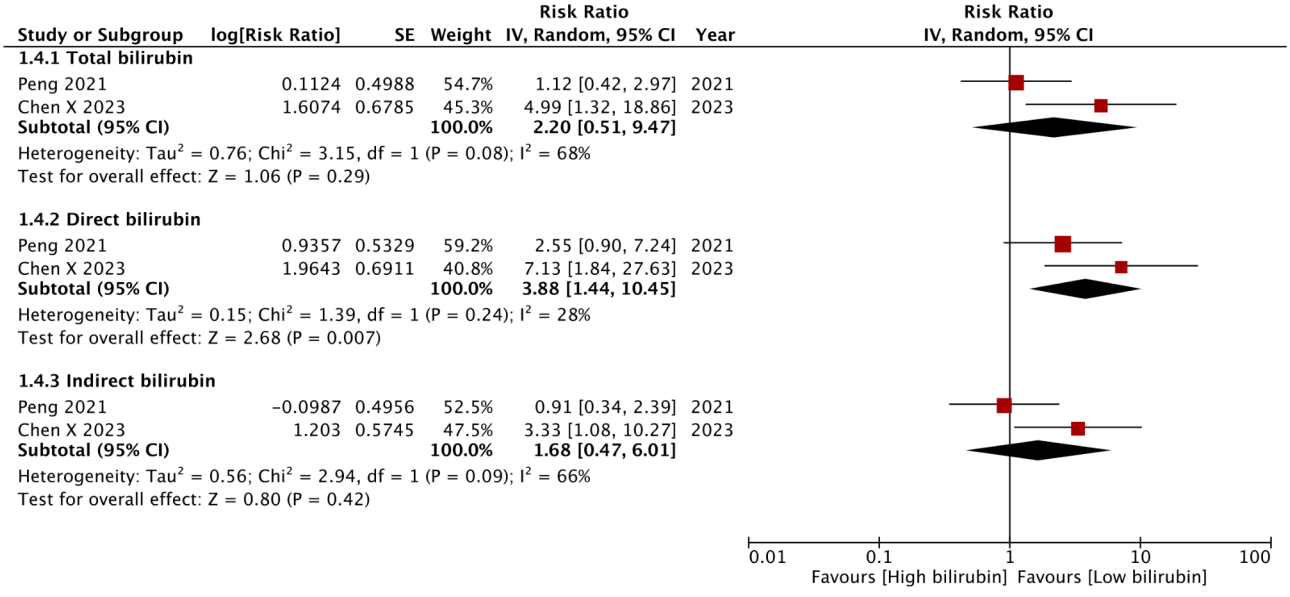

Fig. 6 Meta-analysis of sICH after AIS based on serum bilirubin levels as a categorical variable

for up to 4 h increased the number of caveolae and levels of caveolin-1 and vascular endothelial growth factor. Still, longer exposure (72 h) resulted in disruption of tight junctions and cell-to-cell contacts, thereby damaging the integrity of the blood-brain-barrier [44]. This may explain the risk of haemorrhagic transformation, sICH, and subsequent poor functional outcomes in AIS. Thus, bilirubin may have dual effects reflected in its association with the risk and outcomes of AIS. The duration and levels of bilirubin exposure may constitute important confounders that require further research.

The limitations of our review need to be stressed before concluding. The quantity of studies included in the review was relatively low. Data on functional outcomes and mortality were too scarce for a meta-analysis. Additionally, there was variability in data reporting, outcome definitions, and differences in bilirubin cut-offs, factors which precluded a thorough subgroup analysis. Most studies included did not segregate outcomes based on

treatment and hence we could not assess whether outcomes differed due to management modalities. We also acknowledge the high heterogeneity in certain analyses of the review. We believe that variations in patient populations, baseline characteristics, stroke severity, treatment protocols, bilirubin cut-offs, etc. could have contributed to the high heterogeneity in the meta-analysis. It is recommended that the results of meta-analyses with high heterogeneity must be interpreted with caution. Lastly, the predominance of Chinese data is another drawback. Evidence from other regions is needed before generalizing our conclusions.

To the best of our knowledge, ours is the first review examining the association between bilirubin levels and AIS outcomes in detail. We restricted our review to only adjusted data to eliminate at least some confounding factors. We conducted a detailed review of several outcomes to present the best available evidence.

We believe that more robust future studies especially from countries other than China will help improve the quality and generalizability of evidence. Future studies should report all important stroke outcomes like stroke severity, mortality, functional outcomes, sICH, and haemorrhagic transformation to allow a detailed assessment of the prognostic ability of bilirubin. Lastly, studies should standardize cut-offs of bilirubin to enhance comparability across studies and increase the clinical applicability of bilirubin as a prognostic marker. Studies should also report the accuracy of bilirubin to predict outcomes with these cut-offs. This would help clinicians to have a reliable prognostic assessment leading to improved treatment decisions and better overall patient care.

Conclusions

Serum bilirubin levels are positively associated with the severity of AIS. The evidence suggests that bilirubin levels may be a potential indicator of haemorrhagic transformation and sICH after AIS. Further investigations are required to clarify the association between bilirubin levels and mortality/functional outcomes after AIS.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04168-7.

Supplementary Material 1

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Conception and design: YZ. Data collection and Analysis and interpretation of data: YZ and LL. Writing, review, and/or revision of the manuscript: YZ.

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Data availability
The authors confirm that the data supporting the findings of this study are available within the article and in its supplementary materials.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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