

# Serum bilirubin and ischaemic stroke: a review of literature

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## ABSTRACT

Bilirubin, a product of heme metabolism, is the most potent endogenous antioxidant which increases in many oxidative stress conditions such as stroke. It has been widely known to exert neuroprotective effect on stroke through mechanisms involved in development, therefore, it can influence the occurrence and prognosis of ischaemic stroke (IS). In this review, studies were identified by a comprehensive search of Pubmed, Embase, the Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Methodology Register) and Web of Science to examine the correlation between serum bilirubin levels and risks of developing IS as well as IS outcomes. Additional studies were identified by reviewing references and contacting authors.

## INTRODUCTION

Stroke is the second-leading cause of mortality and the leading cause of long-term disability worldwide.<sup>1</sup> Once ischaemia occurs, excessive oxidative stress ensues and leads to structural and functional damage to the brain, which plays an important role in the pathogenesis of ischaemic brain damage, especially throughout the acute phase of ischaemic stroke (IS).<sup>2–4</sup> Compared with other organs, human brain has few sources of endogenous antioxidants,<sup>5</sup> which poses it more vulnerable to oxidative injury. Recently, a number of therapeutics with antioxidants have shown encouraging results in acute ischaemic stroke (AIS).<sup>6</sup> Bilirubin, a compound used to diagnose conditions such as hepatobiliary disorders,<sup>7</sup> haemolytic anaemia<sup>8</sup> and dyserythropoiesis,<sup>9</sup> also known as a potential toxic factor causing severe brain damage in newborns,<sup>10</sup> is not only the end product of the heme catabolic pathway but also the most potent endogenous antioxidant.<sup>11</sup> It has been shown to protect from diverse diseases associated with increased oxidative stress, such as IS.<sup>12</sup> In addition, accumulating evidence has shown that bilirubin harbours anti-inflammatory,<sup>11</sup> neuroprotective<sup>13</sup> and platelet activation inhibiting<sup>14</sup> effects. In this review, we have summarised available evidence regarding the correlation between circulating bilirubin levels and risks of IS as well as the prognosis of AIS in order to verify the usefulness of

bilirubin serving as a biomarker for stroke occurrence and prognosis.

## SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified in databases of PubMed, Embase, the Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Methodology Register) and Web of Science up to August 2019, and from reference lists of relevant articles. Combinations of searching terms were “stroke” or “cerebr\* vascular disease”, or “cerebr\* ischaemia”, or “intracerebr\* haemorrhage”, or “cerebr\* haemorrhage”, or “brain isch\*”, or “brain haemorrhage” and “bilirubin”, or “bilirubin\*” and “outcome\*”, or “prognos\*”, or “predict\*”, or “mortality”, or “death”, or “dependenc\*”, or “disability”, or “neurological deterioration”, or “functional depend\*”. Language was limited to English. Titles and abstracts were examined for their relevance to the present review, and basic science articles were included if their methods were considered to be of high quality. Articles were excluded if their full texts were unavailable. Relevant prior and ongoing clinical trials investigating the relationship between levels of serum bilirubin and IS outcomes were also included. Results of the study-selection process were shown in figure 1.

## PROPOSED POSSIBLE MECHANISMS OF NEUROPROTECTION

Blockage of cerebral vessels, either temporarily or permanently, triggers a complex series of neurochemical processes including neuronal excitotoxicity, oxidative stress, production of free radicals, blood-brain barrier dysfunction, lipid peroxidation and finally cell death of neurons, glial and endothelial cells.<sup>15</sup> Oxidative stress in the acute phase of IS augments brain injury when the production of free radicals exceeds the endogenous scavenging capacity of the intracellular antioxidation defense system.



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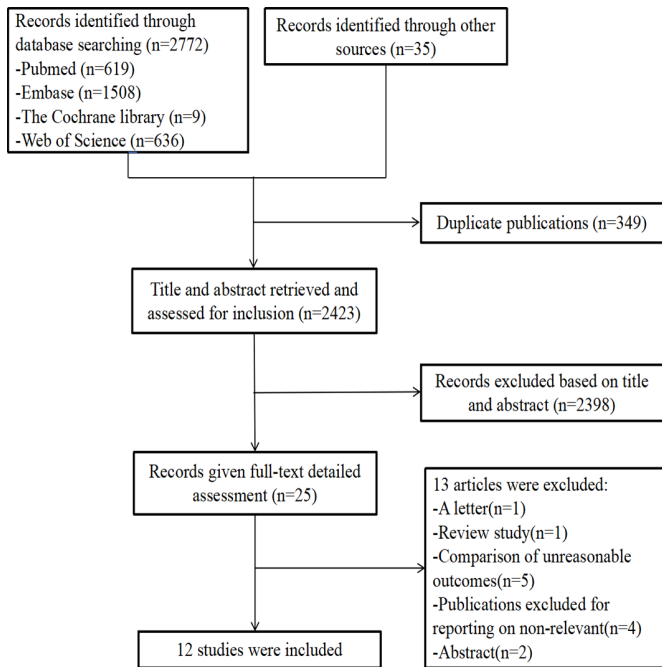
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**Figure 1** Flow chart of studies included.

Bilirubin, the most potent endogenous antioxidant, is rapidly increased in conditions of oxidative stress,<sup>16</sup> such as brain ischaemia. Studies have confirmed that the body can produce a large amount of bilirubin on the stimulation of tissue hypoxia, free radicals and proinflammatory cytokines. Interestingly, reduction of antioxidants, such as glutathione, in vivo can also increase the production of bilirubin.<sup>17</sup> Increased bilirubin levels may be a compensatory mechanism protecting the brain from ischaemia. In an animal study, Vasavda *et al* found that the redox activity of bilirubin was particularly important in the brain where it prevents excitotoxicity and neuronal death by scavenging superoxide during N-methyl-D-aspartic acid neurotransmission.<sup>18</sup> Earlier studies support that bilirubin exerts effects not only through a direct antioxidant pathway as an antioxidant, but also through an indirect pathway. The latter was completed by inhibiting the activity of the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase with tetrapyrroles.<sup>19–21</sup> Other studies observed that moderately hyperbilirubinemic mice exhibited less nitrositive/oxidative stress, confirming that bilirubin indirectly acts by inhibiting the activity of NADPH oxidase.<sup>22–23</sup> These findings supported that bilirubin is a powerful free radical scavenger that can effectively prevent neuronal damage caused by ischaemia and may be a potential therapeutics for IS.

Recently, it was proposed that bilirubin played an important role in immunosuppression and inhibition of protein phosphorylation through modulating intracellular signalling pathways, implying its involvement in vascular and autoimmune pathologies.<sup>24</sup> Vermeer *et al* reported that increase in bilirubin levels due to vitamin B12 deficiency augmented the risk of developing stroke

by increasing levels of homocysteine.<sup>25</sup> These findings provide new insight into the importance of bilirubin, raising the possibility that modulating the level or activity of serum bilirubin could be a novel way to alleviate atherosclerosis, autoimmunity and neurodegenerative conditions.

## LEVELS OF SERUM BILIRUBIN AND RISKS OF ISCHAEMIC STROKE

Studies have demonstrated that the level of bilirubin may serve as a predictor of some vascular events, such as hypertension,<sup>26</sup> coronary artery diseases,<sup>27</sup> diabetes mellitus (DM),<sup>28</sup> diabetic kidney disease,<sup>29</sup> metabolic syndrome,<sup>30</sup> peripheral artery disease<sup>31</sup> and carotid atherosclerosis,<sup>32</sup> which are vascular risk factors of IS. Other studies also further analysed the impact of bilirubin metabolism-related diseases on stroke risk. Jørgensen *et al* found that 221 out of 9742 overweight/obese patients with high risks of cardiovascular diseases (CVD) developed strokes. It was observed from these stroke patients that low levels of bilirubin were associated with an increased risk of stroke.<sup>33</sup> A large Chinese cross-sectional survey found that a low level of total serum bilirubin (TBIL) was associated with an increased risk of stroke in patients with type 2 DM.<sup>34</sup> Patients who had bilirubin metabolism-related diseases and low levels of TBIL may have a higher risk of stroke than those who had high levels of TBIL. Multifaceted interventions achieving the blood pressure (BP), lipid and glycaemia control targets may reduce the risk of developing CVD associated with low levels of bilirubin.

Nowadays, the relationship between the level of serum bilirubin and the risk of developing IS is becoming a hot topic. A large cross-sectional study in the USA showed that an increment of total bilirubin by 1.71  $\mu\text{mol/L}$  reduced the incidence of IS by 9% among 13 214 adult participants.<sup>35</sup> In a meta-analysis of four population-based prospective studies with 2641 incident stroke cases enrolled, the pooled multivariate-adjusted relative risk (95% CI) for stroke was 0.93 (0.88 to 0.98;  $p=0.006$ ) per 1-SD increase in the level of total bilirubin.<sup>36</sup> In a prospective cohort study consisting of 90 532 participants who underwent health examination, Yang *et al* found that the level of TBIL was an independent risk factor of new cerebral infarcts when its level was in the range of 1.0–9.7  $\mu\text{mol/L}$ .<sup>37</sup> A meta-analysis study indicated that 10.0  $\mu\text{mol/L}$  TBIL was a clear cut-off point for discriminating high from low cardiovascular risks.<sup>38</sup> Accumulating evidence from experimental studies involving models of allograft rejection, ischaemia-reperfusion injury, inflammation and arteriosclerosis suggests that bilirubin is emerging as a ‘natural’ protectant that can be used to prevent/treat atherosclerosis related diseases,<sup>39</sup> such as IS.

However, a small number of studies showed only moderately positive or null relationship between the level of bilirubin and the risk of stroke.<sup>40–41</sup> Moreover, a

number of studies showed that the correlation between bilirubin and IS was associated with gender. Kimm *et al* found that every 1  $\mu\text{mol/L}$  increment in bilirubin concentration was associated with 2% reduction in the HR in a multivariate-adjusted model for IS in men and 1% reduction in the HR for all types of stroke in a prospective cohort study in which 1964 out of 78 724 developed stroke.<sup>42</sup> A meta-analysis of 11 population-based observational studies involving 5060 stroke cases among 131 450 subjects showed an inverse relationship between the level of TBIL and the risk of IS in males, but not in females.<sup>12</sup> The difference in the level of serum bilirubin between males and females might be attributed to differences in the level of serum oestrogen, iron storage, heme oxygenase and lifestyle (drinking, smoking, diet and antioxidant supplements (such as vitamin C)).<sup>43–45</sup>

IS can be categorised into different clinical subtypes. In a cross-sectional study, 628 consecutive patients with AIS were classified according to the TOAST aetiological categorisation, and it was found that TBIL was an independent predictor of cardioembolic stroke (SCE).<sup>46</sup> Tan *et al* showed that bilirubin was also positively related to SCE but not independently.<sup>47</sup> There are two possible explanations: first, the level of bilirubin reflects the intensity of oxidative stress after stroke, and it could be higher in SCE than in other subtypes of stroke due to its more prominent severity<sup>3</sup>; second, bilirubin was positively related to heart diseases associated with SCE.<sup>27</sup> Contrarily, Tan *et al* also reported a linear inverse relationship between the incidence of large-artery atherosclerosis (LAA), small-artery occlusion, stroke of undetermined aetiology and the elevation of quartiles in bilirubin according to the TOAST aetiological categorisation.<sup>47</sup> In the above-mentioned conditions, long-standing and progressive pathological changes in the responsible vessel and nearby neurovascular unit were observed, which were correlated with inflammation, oxidative stress and atherosclerosis.<sup>48 49</sup> A mild increase in bilirubin could, therefore, stop the progression of these pathological changes and prevent the occurrence of non-SCE. In a cross-sectional study involving 2865 subjects undergoing medical check-up, Li *et al* reported that a higher level of TBIL was associated with a lower risk of silent cerebral infarction.<sup>50</sup> The latter condition showed similar pathogenesis to lacunar infarcts and both of these conditions displayed endothelial dysfunction.<sup>51</sup>

### SERUM BILIRUBIN AND ISCHAEMIC STROKE OUTCOMES

A meta-analysis published in 2019 suggested that the level of TBIL was an important predictor for the long-term prognosis of vascular-related diseases<sup>52</sup> though conclusions from relevant studies were conflicting.

#### Higher bilirubin levels were associated with greater stroke severity and poorer functional outcomes

Arsalan *et al* found that acute stroke patients with higher levels of serum bilirubin had greater stroke severity (the

National Institutes of Health Stroke Scale score, NIHSS score) and poorer functional outcomes (the modified Rankin Scale score, mRS score) during hospitalisation and at discharge.<sup>53</sup> Kurzepa *et al* reported that the level of TBIL in the acute phase of IS proved to be a bad prognostic factor not only for early neurological status (NIHSS score) but also for long term neurological functions (Barthel Index score) measured 3 months after stroke onset.<sup>54</sup> From 73 patients with LAA stroke, Wang *et al* found that the level of TBIL (direct bilirubin (DBIL), indirect bilirubin) was positively associated with stroke severity (NIHSS score) on day 1, 7 and 14, as well as with poor functional outcomes (mRS score) on day 30.<sup>55</sup> Other studies also showed that both levels of serum DBIL and TBIL were positively correlated with stroke severity<sup>3 56–58</sup> and poor prognoses<sup>59 60</sup> in AIS. Another study reported that patients with higher DBIL levels had significantly greater NIHSS scores on admission than those with lower levels of DBIL, whereas TBIL did not show this type of correlation.<sup>61</sup> Possible reasons for this discrepancy were as follows: first, the sample size of this study is small and the relationship between levels of serum TBIL and stroke severity is less likely to be discovered; second, a number of studies have suggested that the level of DBIL might be a better biomarker for prognosis than the level of TBIL for individuals with general medical conditions.<sup>62–64</sup>

#### Higher serum total bilirubin level is associated with improved stroke outcomes

Perlstein *et al* reported that a 1.71  $\mu\text{mol/L}$  increment in the level of TBIL was associated with a 10% decrease in the odds of an adverse stroke outcome in subjects with a history of stroke.<sup>35</sup> Not surprisingly, three studies (Pineda *et al*, Xu *et al* and Bhatia *et al*) showed no significant relationship between levels of serum bilirubin and short-term clinical outcomes among AIS patients.<sup>58 61 65</sup> Currently, no studies have explored the impact of bilirubin metabolism-related diseases on stroke outcome.

#### Possible reasons for the above-mentioned discrepancy

Bilirubin is a metabolic end product of heme degradation by heme-oxygenase (HO) and exhibits both neuroprotective and neurotoxic effects. Survival of neurons and glial cells relies on a low concentration of unconjugated bilirubin which inhibits the oxidation of linoleic acid and phospholipids and removes free radicals. However, a high concentration of unconjugated bilirubin can be cytotoxic, increasing the permeability of the mitochondrial membrane, damaging mitochondrial function as well as decreasing the activity of astrocytes. As a result, increased neurocyte apoptosis was observed.<sup>55 66</sup> Therefore, pathological levels of bilirubin serve as an indicator of severe brain injury and poor prognosis, whereas high levels of bilirubin in the normal range might be an indicator of good neurological outcome. It was proposed that stroke severity at admission was a strong confounding factor influencing

**Table 1** Relationship between serum bilirubin and ischaemic stroke outcomes

Outcomes reported						
Study	Outcome 1		Outcome 2		Outcome 3	
	Unit	Follow-up point (days)	Unit	Follow-up point (days)	Unit	Follow-up point (days)
Bhatia <i>et al</i> <sup>65</sup>	Mortality	30				
Perlstien <i>et al</i> <sup>65</sup>	Adverse stroke outcomes	1				
	A 'Physical Function' questionnaire					
Pineda <i>et al</i> <sup>61</sup>	Neurological impairment	1	Death or disability	1	mRS	At discharge
	NIHSS score					mRS score >3
Kurzepa <i>et al</i> <sup>64</sup>	Functional disability	90	Neurological impairment	10	NIHSS score	1, 3, 5 and 10
Arsalan <i>et al</i> <sup>63</sup>	Neurological impairment	7.25 (at discharge)	Death or disability	7.25 (at discharge)	mRS	7.25 (at discharge)
Luo <i>et al</i> <sup>67</sup>	Neurological impairment	1	Relative severe stroke	1	NIHSS score	≥8
Luo <i>et al</i> <sup>63</sup>	Neurological impairment	1	Relative severe stroke	1	NIHSS score	≥8
Markaki <i>et al</i> <sup>60</sup>	Death	28 months				
Xu <i>et al</i> <sup>68</sup>	Neurological impairment	1	Higher severity	1	NIHSS score	≥10
Ademiluyi <i>et al</i> <sup>66</sup>	Neurological impairment	1	Severe stroke	1	NIHSS score	≥14
Sagheb Asl <i>et al</i> <sup>69</sup>	Death	14				
Wang <i>et al</i> <sup>65</sup>	Neurological impairment	1, 7 and 14	Disability	30	mRS	mRS score >3

AIS, acute ischaemic stroke; BI, Barthel Index; DBIL, direct bilirubin; IBIL, indirect bilirubin; LAA, large-artery atherosclerosis; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; TBIL, total serum bilirubin.

Bilirubin did not differ significantly in the survivors and expired stroke patients.

Higher TBIL level is associated with improved stroke outcomes.

Higher DBIL level is associated with greater stroke severity but not functional outcome among ischaemic stroke patients.

Serum bilirubin levels are poor prognostic factors for ischaemic stroke.

Higher serum bilirubin levels were associated with increased stroke severity, longer hospitalisation and poor prognosis.

Serum bilirubins were in significant correlation with severity of AIS.

The serum levels of DBIL and TBIL were increased after AIS, which linked to the severity of stroke.

Bilirubin is an independent predictor of mortality in ischaemic cerebrovascular disease patients (ischaemic stroke and transient ischaemic attack).

Serum bilirubin levels were associated with initial stroke severity closely but not short-term clinical outcomes among AIS patients.

AIS patients with higher physiological range of serum bilirubin had more severe stroke (higher NIHSS values).

TBIL, DBIL and IBIL levels were significantly associated with mortality in AIS patients.

Hyperbilirubinaemia might be a biomarker for a poor prognosis in the early identification of LAA strokes.

the prognosis of stroke patients,<sup>67</sup> suggesting that initial stroke severity may be a mediator of the relationship between the level of serum bilirubin and the prognosis of IS patients. Based on these findings, it is possible that a high level of bilirubin might be associated with improved stroke outcome<sup>35</sup> after excluding stroke patients with liver dysfunction, adjusting for alcohol intake and initial stroke severity.<sup>53–55 59 60</sup>

It can be seen from above studies (table 1) that the prognostic value of serum bilirubin in AIS seems still controversial. However, the majority of them appear to support that an elevated level of serum bilirubin is an independent predictor of greater stroke severity and poorer functional outcome after AIS. As a physiological antioxidant, the production of bilirubin was increased in response to oxidative stress and they interacted with each other. The more severe the stroke is, the higher the level of oxidative stress is.<sup>68</sup> Both of them increase the level of serum bilirubin, the most potent endogenous antioxidant.<sup>69</sup> The level of serum bilirubin might be a marker of oxidative stress after AIS. Increased levels of bilirubin are associated with a higher level of IS severity, and the latter in turn results in poorer functional outcomes and increased mortality in AIS patients.

## SUMMARIES AND PERSPECTIVES

The emerging roles serum bilirubin plays in IS as reviewed above imply that the mechanisms through which bilirubin influences IS before and after stroke onset are different. A large number of studies support the notion that bilirubin is involved in antioxidation defense mechanisms and a higher level of serum bilirubin in the normal range was associated with a decreased risk of IS. However, these findings are based on prestroke situations. After stroke occurs, the negative influence of high levels of serum bilirubin on patients' outcome possibly reflects the intensity of initial oxidative stress. AIS patients with higher levels of bilirubin had larger cerebral infarcts, more prominent brain oedema and more severe reperfusion injuries with poorer functional outcomes than those with lower bilirubin levels.<sup>55</sup> On the other hand, elevated bilirubin can protect neurons from oxidative stress injury within a certain concentration range, and the underlying mechanism has been well illustrated previously. Experimental evidence has shown that serum bilirubin seems to account for neuroprotection offered by HO,<sup>70–72</sup> because the neurotoxic effect of HO deletion is reversed by restoring even low concentrations of bilirubin.<sup>71</sup>

However, the specific mechanism bilirubin uses in IS is still unclear. Studies on the relationship between serum bilirubin and prognosis of IS have not reached a consistent conclusion. This also applies to the relationship between levels of serum bilirubin and risks for IS in bilirubin metabolism-related diseases (such as hypertension, coronary artery disease, metabolic syndrome and so on). Whether multifaceted intervention achieving

the BP, lipid and glycaemia control targets can attenuate the increased risk of IS associated with low BIL is uncertain. These issues need to be further explored.

**Correction notice** This paper has been revised since first published. Affiliation of author Ping Zhong was amended to 'Department of Neurology, Shanghai Traditional Chinese and Western Medicine Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China.'

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