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# Effect of metformin on the clinical outcomes of stroke in patients with diabetes: a systematic review and meta-analysis

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# Tittle page

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# Effect of metformin on the clinical outcomes of stroke in patients with diabetes: a systematic review and meta-analysis


## Abstract

Background: Stroke are major causes of death and disability globally, especially among diabetic patients. In this study, we aim to scrutinize the effects of metformin on the clinical outcomes of stroke in diabetic patients.

Methods: We systematically searched PubMed, Embase and Web of Science databases for studies published until December 5, 2023. Two authors independently performed a screening of included articles by reviewing titles, abstracts and full text. Any discrepancy between two authors was resolved by consensus or by consulting a third author. Findings were reported as odds ratio (OR) and 95% confidence interval (CI). All statistical analyses were performed using R software version 4.0.2, and a two-sided P value of 0.05 or less was considered statistically significant.

Results: A total of 11 studies involving 18525 participants were included in this review. Pooled analysis has been demonstrated that pre-stroke metformin use could reduce the risk of poor course after stroke by 34% in DM patients (OR =0.66, 95% CI: 0.61-0.72), and reduce the risk of death by 43% (OR = 0.57, 95% CI: 0.51-0.64).

Conclusion: Pre-stroke metformin use is beneficial for the improvement of clinical outcomes in stroke patients with DM, although the potential bias should be carefully considered.

Keywords: Stroke, Diabetes, Meta-analysis, Metformin, Clinical outcomes

## Introduction

Stroke, ischemic or hemorrhagic, is one of the primary causes of mortality and morbidity in the world[1]. Globally, the annual number of strokes and deaths due to stroke increased substantially from 1990 to 2019, particularly among people older than 70 years[2]. An estimated 17.8 million adults in China had experienced a stroke in 2020, with 3.4 million experiencing their first-ever stroke and another 2.3 million dying as a result[3]. In Europe, the prevalence of stroke was 9.2%, and the incidence was 191.9 per 100000 person-years[4]. In the United States, stroke mortality trends increased by 0.5% annually from 2012 through 2020 based on the national mortality data[5]. Despite advances in therapy, the clinical outcome for patients with stroke is still unfavorable. A large prospective observational study showed that the 5-year mortality rate after stroke was 51.7%[6]. The in-hospital mortality was 1.9% for stroke inpatients, and the 12-month fatality rate was 8.6%[7]. In light of this, it is crucial to identify in advance neuroprotective agents which can reduce neurological severity and improve clinical outcomes in stroke.

Disorders of glucose metabolism, highly prevalent and growing worldwide, are well-recognized risk factors for stroke, including type 1 and 2 diabetes mellitus (DM) and pre-diabetes[8]. These disorders are very common among stroke patients: 28% have pre-diabetes and 25% to 45% have DM[9]. Additionally, an association between DM and increased mortality, length of hospital stay, poorer functional outcomes after

stroke has also been demonstrated[10-13]. To decrease the disparity between stroke patients with DM and without, many attentions, to date, were paid to the influence of anti-diabetic agents on the severity of stroke and acute-phase outcomes in DM patients. Metformin, the first-line anti-diabetic drug, improves energy metabolism, and reduces oxidative stress, leading to improved balance of survival and death signaling in neurons[14]. A meta-analysis included 21 studies with 1392809 patients demonstrated that metformin monotherapy is effective in reducing stroke risk, but combined administration of metformin with other antihyperglycemic agents have no significant effect on stroke prevention in DM[15]. Besides serving as the protective factors of stroke, metformin may also be related with the clinical outcomes of stroke. Animal experiments showed that metformin plays a neuroprotection role in stroke and improves clinical outcomes triggered by stroke[16, 17]. In recent years, clinical studies have examined the effects of metformin in stroke outcomes, with some evidence that metformin pretreatment is associated with less severe strokes, improved functional outcome and lower mortality[18, 19]. In contrast, several studies showed that metformin use is not associated with in-hospital mortality and 1-year prognosis in diabetic ICH patients[20, 21]. In the context of existing inconsistencies between studies, the benefits of pre-stroke metformin use for improving the clinical outcome of stroke are continuing controversial.

In order to obtain insight into the issue mentioned above, we, in this study, searched for relevant published studies and performed a meta-analysis to scrutinize the effects

of metformin on stroke outcomes.

## Methods

This study was processed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines and was prospectively registered on PROSPERO (CRD42024496056).

### Literature search strategy

We systematically searched PubMed, Embase and Web of Science databases for studies published until December 5, 2023. The search strategy divided by each database is provided in supplementary material 1. In addition to database searches, we hand-searched the reference sections of included studies in the full-text review and undertook forward and backward citation tracking to find further eligible studies. All the search results were imported into Endnote (X9) and duplicated, if any, were removed.

### Eligibility criteria and study selection

The exposure of interest was the pre-stroke metformin use, and the primary outcome was clinical outcome of stroke. Studies meeting the following criteria were included in this study: (1) reported the effect of pre-stroke metformin use on the outcomes of patients with stroke; (2) included patients with diabetes; (3) the sample size was beyond 10; (4) the report was not a review, comment, case report or letter; (5) full-text articles were available, with no limit to study type. We did not place limitations on its country of origin, nor did we limit the age or gender of the included patients.

**Data extraction**

Firstly, two authors independently performed a screening of articles by reviewing titles and abstracts. Secondly, the full text of potentially eligible articles was retrieved, and relevant articles were assessed based on inclusion criteria. Any discrepancy between two authors was resolved by consensus or by consulting a third author. The following data from included studies were extracted: first author, study title, publication year, country, study design, sample size, patient demographics (i.e., gender distribution and mean/median age), and clinical outcomes (functional outcome and death). Where articles reported the outcome on multiple timepoints, the longest follow-up one was selected. The functional outcome after stroke were graded using a modified Rankin Scale (mRS) score ranging from 0 (no symptoms) to 6 (death). The mRS score was used to classify functional outcome as good course (score of 0 to 2) or poor course (score of 3 to 6).

**Quality assessment**

The quality of each included study was assessed using an eight-items modified version of the Newcastle-Ottawa Scale (NOS) for observational studies[22]. This scale estimates the quality of each study through three perspectives: the selection of sample; the comparability of groups; the ascertainment of outcome. Two authors independently scored each study on every item in the scale. The higher the score, the better the methodologic quality of the study.

**Statistical analysis**

All statistical analyses were performed using R software version 4.0.2, and a two-sided P value of 0.05 or less was considered statistically significant. Data were recorded as the number of events in metformin use and non-metformin use groups. The pooled odds ratio (OR) and 95% confidence interval (CI) was calculated. The $I^2$ statistic (significance level was set at $I^2 > 50$ %) and Chi-square test (significance level was set at $P < 0.10$). Also, $I^2$ values of 25 % and 75 % were used as the criteria for classifying the degree of intertrial heterogeneity, namely low heterogeneity ($I^2 <$ 25 %), moderate heterogeneity (25 % $\leq I^2 <$ 75 %), and high heterogeneity ($I^2 \geq$ 75 %). Publication bias was visually assessed using funnel plots, and quantified by the Egger's test. Additionally, sensitivity analysis of the pooled ORs was conducted by omitting 1 study in each turn, to estimate the impact of an individual study on the pooled results. A series of subgroup analysis and meta-regression according to country, publication year, study design, the type of stroke, follow-up duration and sample size were performed to explore the potential source of heterogeneity, and the pooled ORs between subgroups were compared using the Chi-square test.

## Results

### Study characteristics

A flow chart describing the selection of articles identified, included and excluded, with reasons, is presented in Fig. 1. The search in the databases resulted in 1913 non-duplicate articles, 1852 of which were excluded after the screening of the titles and abstracts. The full text of the remaining 51 articles were retrieved and reviewed.

Finally, the data from 10 articles were included in this study[18-21, 23-28]. One study was included through manual review of reference lists[29]. Nine studies reported the functional outcome, and seven articles the survival status. The eligible articles involved a total of 18525 patients, 7317 of which were with pre-stroke metformin use. The articles enrolled patients from a diverse range of geographical locations and ethnical populations. Detailed information on the included studies is summarized and presented in Table 1.

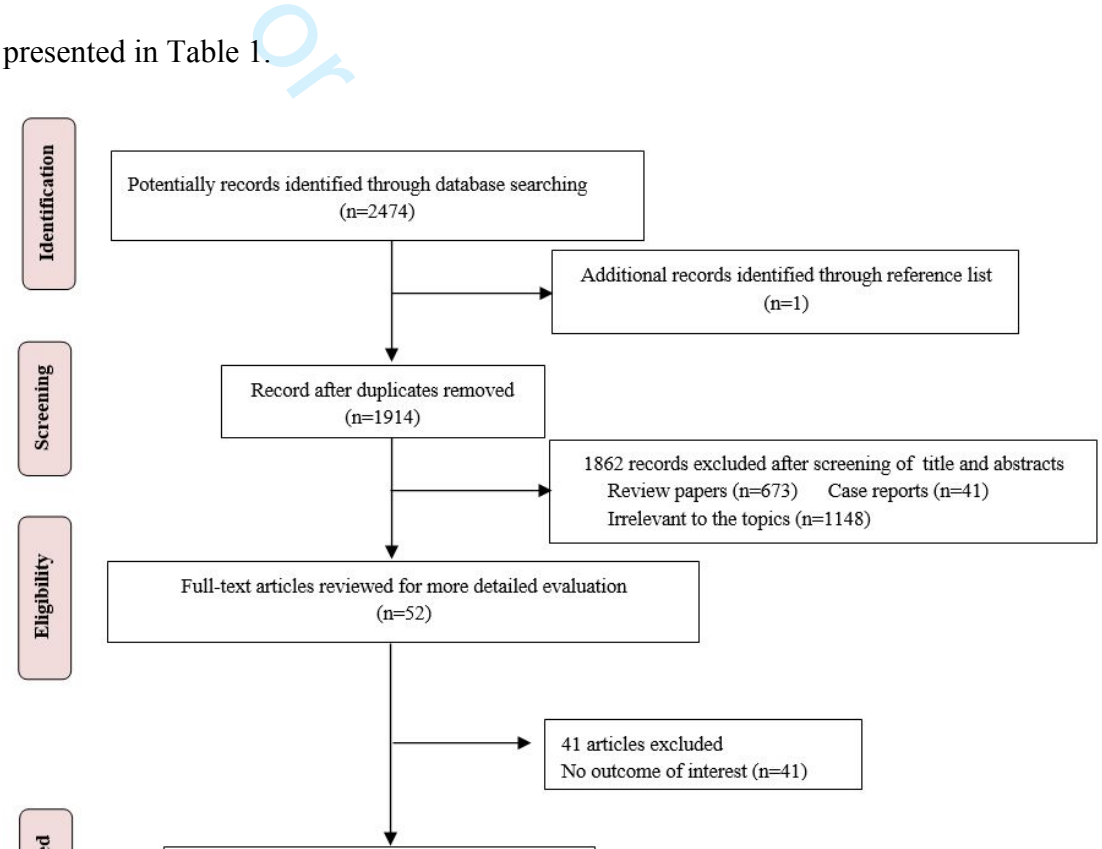


Fig. 1. Flow diagram to illustrate the study selection procedure.

Table 1. Characteristics of included studies

| Author | Year | Country | Sample Size | N (Metformin/ control) | Male (%) | Design | Age | Year collected | Stroke type | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Henriette et.al | 2012 | Denmark | 3841 | 563/3278 | MET+: 305 (54.2) | PCS | MET+: 71.2 (63-79) | 2003-2006 | Ischemic stroke | 30 days, 1 year |
| Takahiro | 2012 | Japan | 241 | 19/222 | 153 (69.0) | RCS | 71±10 | 2007- | Ischemic | 3 months |

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|---|---|---|---|---|---|---|---|---|---|---|
| et.al | | | | | | | | 2008 | stroke | |
| Yohei et.al | 2016 | Japan | 355 | 77/278 | 202 (72.7) | RCS | 70.1±10.6 | 2010-2014 | Ischemic stroke | Discharge |
| Teddy et.al | 2016 | Multiple | 374 | 148/226 | 258 (69.0) | RCS | 68 (60-76) | 2005-2010 | ICH | 90 days |
| Westphal et.al | 2020 | European | 1919 | 757/1162 | MET+: 478 (63.1), MET-: 667 (57.4) | RCS | MET+: 71, MET-: 74 | NR | Ischemic stroke | 3 months |
| Wen-Jun et.al | 2021 | China | 730 | 281/449 | 421 (57.7) | RCS | 65 (56-72) | 2010-2019 | ICH | Discharge, 1 year |
| Naveed et.al | 2022 | Qatar | 2157 | 1132/1025 | 1,752 (81.2) | RCS | 54.5±13.1 | 2013-2020 | Ischemic stroke | Discharge, 90 days |
| Kersten et.al | 2022 | Netherlands | 937 | 592/345 | MET+: 332 (56), MET-: 167 (48) | RCS | MET+: 75 (10), MET-: 76 (11) | 2017-2021 | Ischemic stroke | 3 months |
| Wen-Jun et.al | 2022 | China | 7587 | 3593/3994 | 4351 (57.35) | PCS | 66 (57–73) | 2019-2019 | Stroke | Discharge, 1 year |
| Natsuki et.al | 2023 | Japan | 160 | 55/105 | NR | RCS | MET+: 75, MET-: 73 | 2010-2021 | Ischemic stroke | Discharge |
| Yating et.al | 2023 | China | 224 | 94/130 | MET+: 64 (68.1), MET-: 78 (60.0) | RCS | MET+: 64 (54–71), MET-: 65 (56–74) | 2017-2021 | Ischemic stroke | 90 days |

NR: Not reported; RCS: Retrospective cohort study; PCS: Prospective cohort study.

## Quality assessment

Ten included studies scored seven or above on the NOS checklist (Supplementary material 2) while one study scored six. All patients met inclusion criteria in specific region were consecutively recruited within a certain period, and were divided into two groups according to the metformin or non-metformin use prior stroke, ensuring the representativeness and comparability of groups. The ascertainment of metformin use clearly were described in six studies[18, 21, 26-29]. All including studies had a longer than 3 months follow-up to determine the functional outcome or survival status of the

patients except for two studies reported discharge outcome only[25, 28].

**Effect of pre-stroke metformin use on prognosis of stroke**

The effect of pre-stroke metformin use on the improvement of functional outcome after stroke was assessed in nine cohort studies. Fig 2 shows the comparison of functional outcome between patients with pre-stroke metformin use and patients without, with individual and pooled ORs with corresponding CIs. Individual ORs ranged from 0.49 (95% CI: 0.24-0.97) to 0.87 (95% CI: 0.72-1.05), and pooled analysis showed that pre-stroke metformin use could reduce the risk of poor course after stroke by 34% in DM patients (OR =0.66, 95% CI: 0.61-0.72). The application of Chi-square test and $I^2$ statistic showed that no significant heterogeneity existed among studies ($p = 0.06$, $I^2 = 47\%$), and a fixed-effects model was applied.

Also, seven studies with a total of 15769 patients reported the difference in survival status between patients with or without pre-stroke metformin use. The comparison of survival status between patients with pre-stoke metformin use and without is presented in Fig 3, with individual and pooled ORs with corresponding CIs. Individual ORs ranged from 0.16 (95% CI: 0.02-1.33) to 0.73 (95% CI: 0.47-1.13), and the pooled analysis indicated a 43% reduction in the risk of death after stroke (OR = 0.57, 95% CI: 0.51-0.64). There was also no significant heterogeneity among studies ($p = 0.67$, $I^2 = 0\%$), and a fixed-effect model was performed.

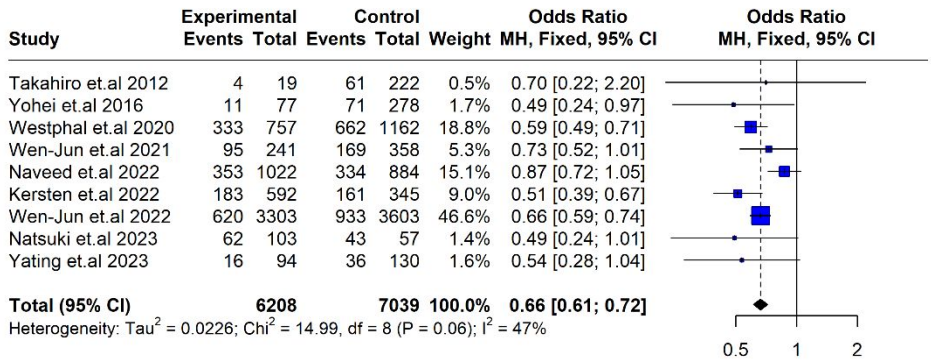


Fig. 2. Forest plot for functional outcome between patients with metformin use and patients without.

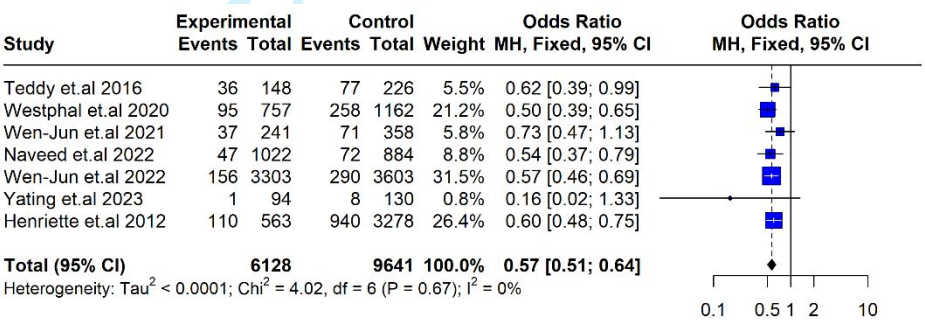


Fig. 3. Forest plot for survival status between patients with metformin use and patients without.

## Subgroup and sensitivity analysis

The results of subgroup analysis showed that there are no significant differences across from different subgroup except for region (Supplementary material 3). Meta-regression was performed for all the two outcomes (functional outcome and survival status). None of the variables were significant or had a $p < 0.20$ for studies reporting the association between metformin use and clinical outcomes after stroke; hence, a multivariable meta-regression was not attempted. The results of sensitivity analysis

showed that the pooled OR was steady, and removing one study did not change the significance of pooled OR. For functional outcome, the pooled OR ranged from 0.63 (0.57-0.68) to 0.68 (0.62-0.74); and for survival status, the pooled OR ranged from 0.56 (0.49-0.64) to 0.59 (0.52-0.67).

**Publication bias**

Publication bias in the included studies was assessed by using Egger's test and funnel plot. The Egger's test indicated that there was no evidence of publication bias for the assessment of the effect of metformin use on functional outcome (p = 0.503) and survival status (p = 0.608). Also, the funnel plots revealed evidence of symmetry for functional outcome and survival status (Supplementary material 4).

**Discussion**

Metformin is a cheap, widely available, safe, and first-line anti-diabetic drug. Recently, metformin has also been demonstrated to be effective in decreasing the risk of stroke in DM patients[15]. In this study, we summarized evidence from published studies for now through a meta-analysis to prove that DM patients with pre-stroke metformin use had a better functional outcome and lower risk of death after stroke compared to those without. Thus, metformin in DM patients may not only be beneficial for reducing the risk of stroke, but also for improving the clinical outcomes after stroke.

As is well-known, hyperglycemia on admission was related with poor outcomes in stroke patients[30, 31], likely mediated through increased risk of infection and cardiac

complications[23, 26, 32]. Pre-stroke glycemic control, as HbA1c level on admission, is a useful way to improve clinical outcomes in DM patients with stroke[33]. Thus, one possible pathway for the protective effect of metformin on stroke is through lowing blood glucose in DM patients. Moreover, interestingly, accumulating evidence showed that although there is no statistically relevant difference between admission glucose levels of metformin group and sulfonylureas group[25], preadmission use of sulfonylureas does not affect stroke severity and clinical outcome among DM patients admitted with stroke[29, 34]. It seems to support the hypothesis of metformin pre-conditioning resulted in benefits besides its hypoglycemic effects.

The effect of metformin on clinical outcome was at least partially driven by the lower stroke severity on admission. The severity on admission was also a known determinant of chronic clinical outcomes after stroke[35-37]. A study, included 1281 patients with stroke, reported that the NIHSS score on admission, reflected the severity of stroke, could strongly predict the functional outcome after stroke, and patients with a score of NIHSS $\geq$ 16 on admission have a higher probability of death or sever disability than those without[37]. Several studies have shown that pre-stroke metformin use may be related with reduced neurological severity in stroke[18, 25]. In a cohort study with a total of 1919 stroke patients, patients with metformin treatment prior to stroke showed less severe strokes demonstrated by a lower NIHSS on admission compared to the non-pretreated patient group[18]. Similarly, a study identified metformin as the only antidiabetic drug to represent a significantly

favorable determinant of stroke severity[25]. These results support the view that metformin may be an active option for DM patients, not only because of its position as a DM treatment, but also because of its neuroprotective effects[28].

One possible mechanism of metformin-induced neuroprotective in stroke is related with neuronal adenosine monophosphate-activated protein kinase (AMPK), an important mediator of cellular energy homeostasis, highly expressed in neurons and activated under low cellular energy conditions, e.g., cerebral ischemia[38, 39]. Studies have demonstrated that AMPK plays a protective role in brain[40, 41]. Evidences from animal experiment showed that metformin, in acute stroke patients with DM, could improve the neurological function and oxidative stress status by the AMPK/mTOR signaling pathway and oxidative stress[42, 43]. However, it is worth noting that the neuroprotective effects require chronic use of metformin. Acute metformin use exacerbated stroke damage, enhanced AMPK activation, and led to metabolic dysfunction. Conversely, chronic metformin use was neuroprotective, improved stroke-induced lactate generation, and ameliorated stroke-induced activation of AMPK[38]. Therefore, the timing and duration of metformin use in DM patients should be taking consideration to achieve neuroprotection. Tian et.al indicated that a pretreatment time window no less than 7 days was required for the neuroprotection of metformin against acute brain injury, and the time window cannot be reduced by increasing metformin dosage[43]. The cumulative dynamics of metformin dosage may be a key of the protective effects for stroke by metformin pretreatment. Additionally,

chronic metformin use after stroke is also beneficial for clinical outcome by inhibiting the inflammatory response, stimulating vascular endothelial growth factor expression and promoting angiogenesis[25]. Thus, metformin was a potential target in therapeutic intervention of stroke[44].

**Strengths and limitations**

To the best of our knowledge, this meta-analysis of effects of pre-stroke metformin use on the clinical outcomes of stroke in DM represents the first and pooled analysis of available evidence on this issue with a large pooled sample size. Nevertheless, although the results in this study are believed to highly stable, some limitations are acknowledged. First, only English studies included in this study, and the quantity of studies included was limited. Second, most of included studies were retrospective, which is likely to increase the risk of confirmation bias, making it difficult to confirm causality. Third, the frequency and duration of metformin use may be influence on the results, but this information was not adequate for consideration in this study, because few included studies provided this information. Whether metformin continues to be used after stroke is also unclear. In future researches, these factors should be considered at length. Therefore, the results on this study should be interpreted with caution.

**Conclusion**

In conclusion, pre-stroke metformin use is beneficial for the improvement of clinical outcomes in stroke patients with DM, although the potential bias should be carefully

considered. Metformin, as a known safety profile, may provide an economical and accessible therapeutic option in DM patients to improve stroke outcomes. Future researches in a large, prospective, randomized controlled trial are warranted to further elucidate the mechanisms underlying these associations and to determine whether metformin use may improve the clinical outcomes after stroke in DM patients.

**Authors contribution**

Study design: JZ and JW, Data gathering: YL, FL and YG, Analysis: JL, Interpreting the results: JL, ZH, YG, Drafting: JL, ZH, Critically revised: All authors.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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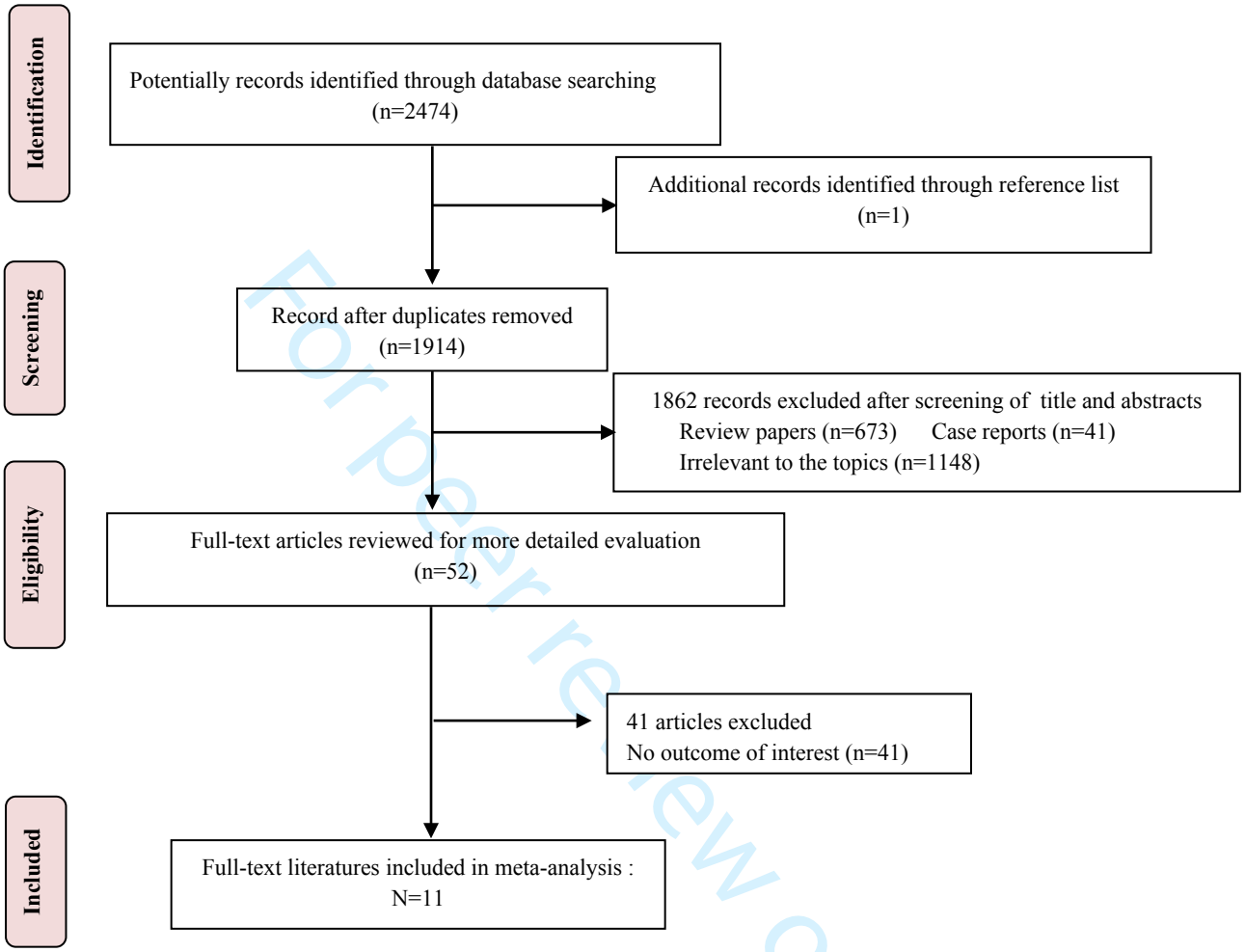


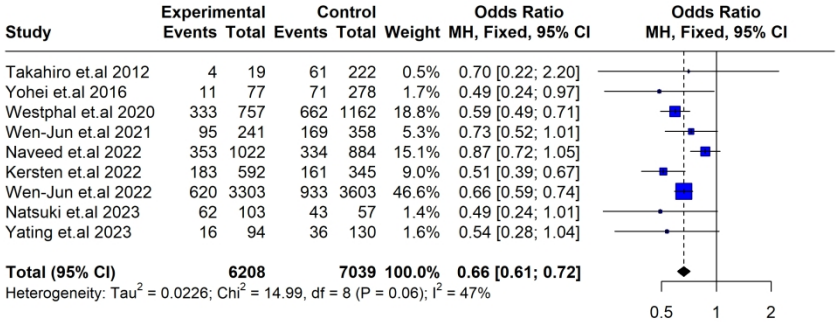



Fig. 2. Forest plot for functional outcome between patients with metformin use and patients without.

254x169mm (300 x 300 DPI)

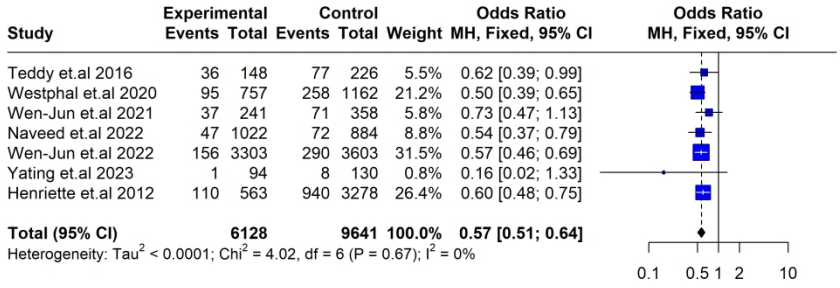



Fig. 3. Forest plot for survival status between patients with metformin use and patients without.

254x169mm (300 x 300 DPI)

**Supplementary material 1: Search terms**

**PubMed**

1. "Metformin"[mh] OR Metformin[tiab] OR Dimethylbiguanidine[tiab] OR Dimethylguanylguanidine[tiab] OR Glucophage[tiab] OR aron[tiab] OR diaformin[tiab] OR dmgg[tiab] OR glucohexal[tiab] OR glucophage[tiab] OR glucophage forte[tiab] OR glucophage retard[tiab] OR glucophage sr[tiab] OR glucophage xr[tiab] OR glucophage xr extended release[tiab] OR glucophagemite[tiab] OR gluformin[tiab] OR glumetza[tiab] OR glyciphage[tiab] OR glycon[tiab] OR i-max[tiab] OR islotin[tiab] OR la 6023[tiab] OR 6023[tiab] OR metiguanide[tiab] OR neoform[tiab] OR nndg[tiab]
2. "Stroke"[mh] OR "Ischemic Stroke"[mh] OR "Embolic Stroke"[mh] OR "Cerebral Infarction"[mh] OR "Infarction, middle cerebral artery"[mh] OR "Brain infarction"[mh] OR "Stroke, Lacunar"[mh] OR "Thrombotic Stroke"[mh] OR Stroke[tiab] OR Cerebral Infarction[tiab] OR Brain infarction[tiab] OR middle cerebral artery infarct*[tiab] OR middle cerebral artery occlusion[tiab] OR Cerebral Infarct*[tiab] OR Brain Infarct*[tiab] OR Hemorrhagic Strokes[tiab] OR Stroke[tiab] OR Cerebrovascular Accident[tiab] OR Cerebrovascular Accident, [tiab] OR Apoplexy[tiab] OR Brain Vascular Accident*[tiab] OR Cryptogenic Embolism[tiab] OR Cerebral Infarct*[tiab] OR Subcortical Infarction[tiab] OR Choroidal Artery Infarction [tiab] OR MCA Infarction[tiab] OR Cerebral Artery Infarction[tiab] OR Cerebral Artery Embol*[tiab] OR Cerebral Artery Occlusion[tiab] OR Cerebral Artery Thromb*[tiab] OR Brain Venous Infarction[tiab] OR cerebral ischemia reperfusion injury[tiab] OR brain ischemi* reperfusion injury[tiab] OR brain ischemia/reperfusion[tiab] OR cerebral ischemia/reperfusion[tiab] OR cerebral reperfusion injury[tiab] OR reperfusion brain injury[tiab] OR acute cerebrovascular lesion[tiab] OR acute focal cerebral vasculopathy[tiab] OR brain vascular accident[tiab] OR cerebrovascular injury[tiab] OR cortical infarction[tiab] OR hemisphere infarct*[tiab] OR hemispheric infarct*[tiab] OR brain

stem infarction*[tiab] OR brainstem infarction[tiab] OR cerebellar infarction[tiab] OR brain ischemia[tiab] OR brain ischaemic attack[tiab] OR brain ischemic attack[tiab]

3. "Treatment outcome"[mh] OR "Treatment outcome"[tiab] OR "outcome"[tiab] OR "outcomes"[tiab] OR "prognosis"[mh] OR "prognosis"[tiab]

**Embase**

1. 'Metformin'/exp OR 'Metformin':ab,ti OR 'Dimethylbiguanidine':ab,ti OR 'Dimethylguanylguanidine':ab,ti OR 'Glucophage':ab,ti OR 'aron':ab,ti OR 'diaformin':ab,ti OR 'dmgg':ab,ti OR 'glucohexal':ab,ti OR 'glucophage':ab,ti OR 'glucophage forte':ab,ti OR 'glucophage retard':ab,ti OR 'glucophage sr':ab,ti OR 'glucophage xr':ab,ti OR 'glucophage xr extended release':ab,ti OR 'glucophage-mite':ab,ti OR 'gluformin':ab,ti OR 'glumetza':ab,ti OR 'glyciphage':ab,ti OR 'glycon':ab,ti OR 'i-max':ab,ti OR 'isotin':ab,ti OR 'la 6023':ab,ti OR 'la6023':ab,ti OR 'metiguanide':ab,ti OR 'neoform':ab,ti OR 'nndg':ab,ti

2. 'cerebral ischemia reperfusion injury'/exp OR 'cerebrovascular accident'/exp OR 'cardioembolic stroke'/exp OR 'brain infarction'/exp OR 'brain stem infarction'/exp OR 'cerebellum infarction'/exp OR 'brain ischemia'/exp OR 'transient ischemic attack'/exp OR 'Stroke':ab,ti OR 'Cerebral Infarction':ab,ti OR 'Brain infarction':ab,ti OR 'middle cerebral artery infarct*':ab,ti OR 'middle cerebral artery occlusion':ab,ti OR 'Cerebral Infarct*':ab,ti OR 'Brain Infarct*':ab,ti OR 'Hemorrhagic Strokes':ab,ti OR 'Stroke':ab,ti OR 'Cerebrovascular Accident':ab,ti OR 'Cerebrovascular Accident, ':ab,ti OR 'Apoplexy':ab,ti OR 'Brain Vascular Accident*':ab,ti OR 'Cryptogenic Embolism':ab,ti OR 'Cerebral Infarct*':ab,ti OR 'Subcortical Infarction':ab,ti OR 'Choroidal Artery Infarction ':ab,ti OR 'MCA Infarction':ab,ti OR 'Cerebral Artery

Infarction':ab,ti OR 'Cerebral Artery Embol*':ab,ti OR 'Cerebral Artery Occlusion':ab,ti OR 'Cerebral Artery Thromb*':ab,ti OR 'Brain Venous Infarction':ab,ti OR 'cerebral ischemia reperfusion injury':ab,ti OR 'brain ischemi* reperfusion injury':ab,ti OR 'brain ischemia/reperfusion':ab,ti OR 'cerebral ischemia/reperfusion':ab,ti OR 'cerebral reperfusion injury':ab,ti OR 'reperfusion brain injury':ab,ti OR 'acute cerebrovascular lesion':ab,ti OR 'acute focal cerebral vasculopathy':ab,ti OR 'brain vascular accident':ab,ti OR 'cerebrovascular injury':ab,ti OR 'cortical infarction':ab,ti OR 'hemisphere infarct*':ab,ti OR 'hemispheric infarct*':ab,ti OR 'brain stem infarction':ab,ti OR 'brainstem infarction':ab,ti OR 'cerebellar infarction':ab,ti OR 'brain ischemia':ab,ti OR 'brain ischaemic attack':ab,ti OR 'brain ischaemic attack':ab,ti

3. 'Treatment outcome'/exp OR 'Treatment outcome':ab,ti OR 'outcome':ab,ti OR 'outcomes':ab,ti OR 'prognosis'/exp OR 'prognosis':ab,ti

**Web of Science**

1. TS=( "Metformin" OR "Dimethylbiguanidine" OR " Dimethylguanylguanidine" OR " Glucophage" OR "aron" OR " diaformin" OR " dmgg" OR " glucohexal" OR " glucophage" OR " glucophage forte" OR " glucophage retard" OR " glucophage sr" OR " glucophage xr" OR " glucophage xr extended release" OR " glucophage-mite" OR " gluformin" OR " glumetza" OR " glyciphage" OR "glyc on" OR " i-max" OR " islotin" OR " la 6023" OR " la6023" OR " metiguanide" OR " neoform" OR " nndg" )

2. TS= ( "Stroke" OR " Cerebral Infarction" OR " Brain infarction" OR " middle cerebral artery infarct*" OR " middle cerebral artery occlusion" OR " Cerebral Infarct*" OR " Brain Infarct*" OR " Hemorrhagic Strokes" OR " Stroke" OR " Cerebrovascular Accident" OR " Cerebrovascular Accident, " OR " Apoplexy" OR " Brain Vascular Accident*" OR " Cryptogenic Embolism" OR " Cerebral Infarct*" OR " Subcortical Infarction" OR " Choroidal Artery Infarction " OR " MCA Infarction" OR " Cerebral Artery Infarction" OR " Cerebral Artery Embol*" OR " Cerebral Artery

Occlusion" OR " Cerebral Artery Thromb*" OR " Brain Venous Infarction" OR " cerebral ischemia reperfusion injury" OR " brain ischemi* reperfusion injury" OR " brain ischemia/reperfusion" OR " cerebral ischemia/reperfusion" OR " cerebral reperfusion injury" OR " reperfusion brain injury" OR " acute cerebrovascular lesion" OR " acute focal cerebral vasculopathy" OR " brain vascular accident" OR " cerebrovascular injury" OR " cortical infarction" OR " hemisphere infarct*" OR " hemispheric infarct*" OR " brain stem infarction*" OR " brainstem infarction" OR " cerebellar infarction" OR " brain ischemia" OR " brain ischaemic attack" OR " brain ischemic attack"

3. TS=("treatment outcome" OR "outcome" OR "outcomes" OR "prognosis")

## Supplementary material 2: Risk bias assessment of included studies

| Study | Item1 | Item2 | Item3 | Item4 | Item5 | Item6 | Item7 | Item8 | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Henriette et.al 2012 | * | * | * | * | * | * | | | 7 |
| Takahiro et.al 2012 | * | * | * | * | * | * | | * | 8 |
| Yohei et.al 2016 | * | * | | * | * | * | | * | 6 |
| Teddy et.al 2016 | * | * | * | * | * | * | | * | 8 |
| Westphal et.al 2020 | * | * | * | * | * | * | | * | 8 |
| Wen-Jun et.al 2021 | * | * | | * | * | * | | * | 7 |
| Naveed et.al 2022 | * | * | | * | * | * | | * | 7 |
| Kersten et.al 2022 | * | * | | * | * | * | | * | 7 |
| Wen-Jun et.al 2022 | * | * | | * | * | * | | * | 7 |
| Natsuki et.al 2023 | * | * | * | * | * | * | | * | 7 |
| Yating et.al 2023 | * | * | * | * | * | * | | * | 8 |

Items:

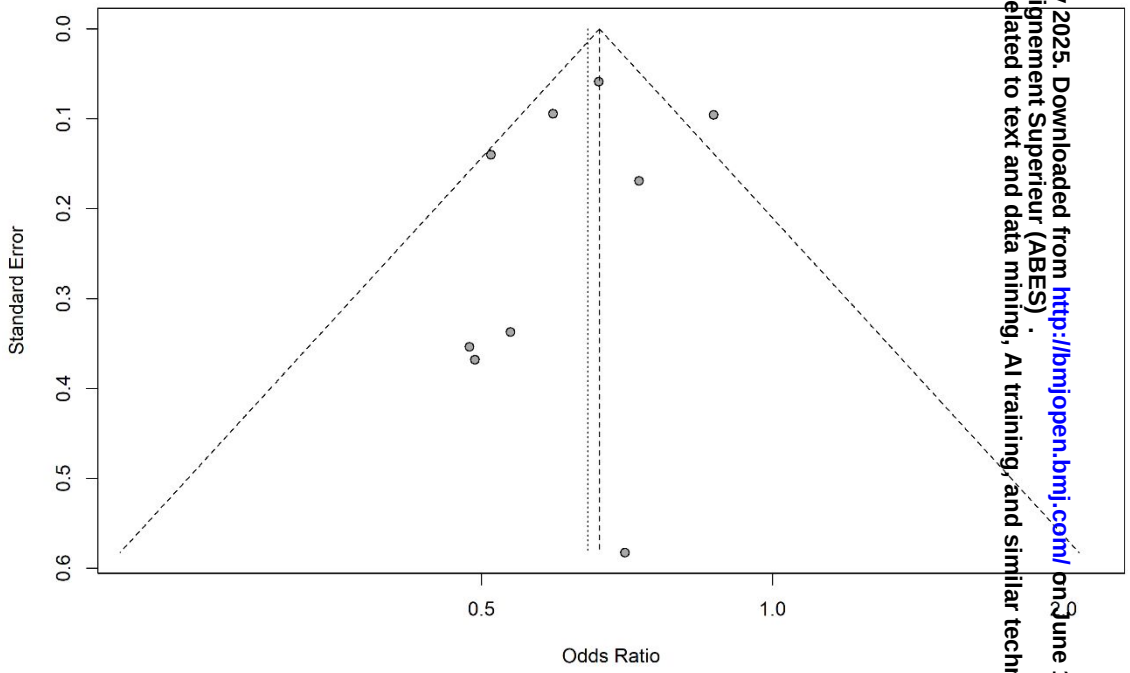
1. Was the exposure cohort representativeness?
2. Was the non-exposed cohort from the same population as the exposed cohort?
3. Was the ascertainment of exposure clearly defined, valid, reliable, and implemented?
4. Was the exposure of interest measured prior to the outcome(s) being measured?
5. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
6. Were the outcome measures clearly defined, valid, reliable, and implemented?
7. Was follow-up long enough for outcomes to occur?
8. Was loss to follow-up after baseline 20% or less?

**Supplementary material 3: Subgroup analysis**

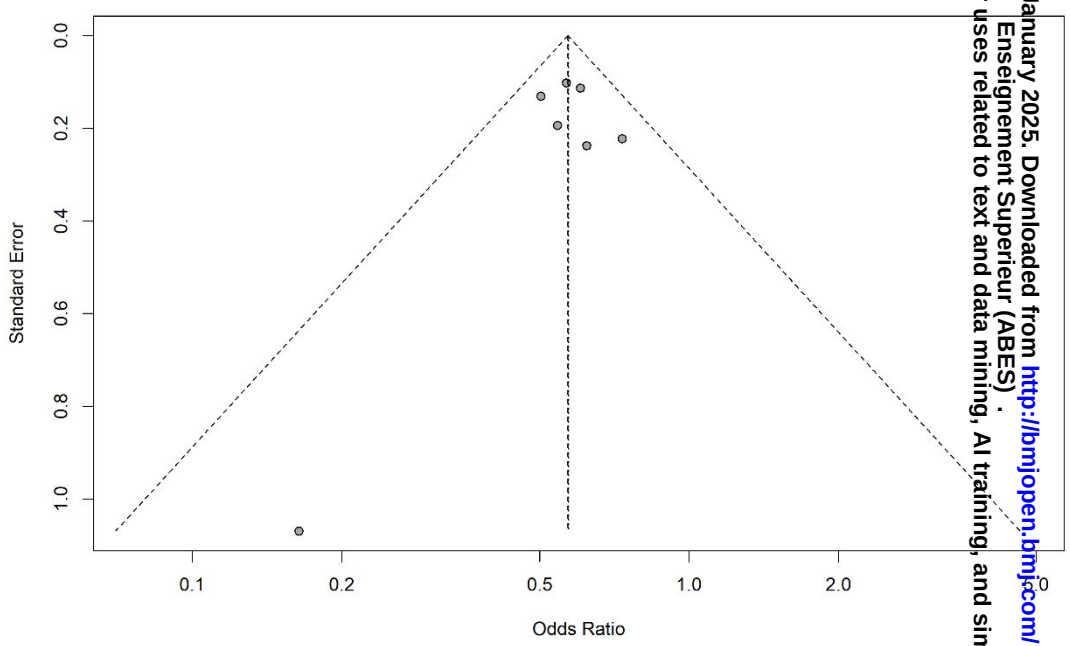
| Subgroup | Functional outcome | Test for subgroup differences | | Survival status | Test for subgroup differences | |
|---|---|---|---|---|---|---|
| | | $Chi^2$ | P | | $Chi^2$ | P |
| Public year | | 2.26 | 0.13 | | 0.02 | 0.88 |
| <=2020 | 0.59 (0.49-0.71) | | | 0.57 (0.48-0.75) | | |
| >2020 | 0.68 (0.61-0.74) | | | 0.58 (0.49-0.68) | | |
| Sample size | | 2.92 | 0.09 | | 0.68 | 0.41 |
| <=1000 | 0.57 (0.48-0.69) | | | 0.65 (0.47-0.88) | | |
| >1000 | 0.68 (0.63-0.75) | | | 0.56 (0.50-0.63) | | |
| Study type | | 0.001 | 0.96 | | 0.22 | 0.64 |
| RCS | 0.66 (0.60-0.74) | | | 0.55 (0.46-0.66) | | |
| PCS | 0.66 (0.59-0.72) | | | 0.58 (0.50-0.68) | | |
| Region | | 13.16 | <0.01 | | 0.31 | 0.96 |
| Japan | 0.52 (0.33-0.82) | | | NA | | |
| European | 0.57 (0.49-0.66) | | | 0.56 (0.47-0.66) | | |
| China | 0.66 (0.60-0.74) | | | 0.58 (0.49-0.70) | | |
| Qatar | 0.87 (0.72-1.05) | | | 0.54 (0.37-0.79) | | |
| Multi-country | NA | | | 0.62 (0.39-0.99) | | |
| Follow-up duration | | 1.46 | 0.48 | | 1.18 | 0.28 |
| Discharged | 0.49 (0.30-0.81) | | | NA | | |
| 3 months | 0.67 (0.60-0.75) | | | 0.52 (0.43-0.63) | | |
| 1 year | 0.67 (0.60-0.74) | | | 0.60 (0.52-0.69) | | |
| Disease type | | 0.33 | 0.85 | | 1.35 | 0.51 |
| IS | 0.66 (0.59-0.73) | | | 0.55 (0.47-0.64) | | |

| | | |
|---|---|---|
| ICH | 0.73 (0.52-1.01) | 0.68 (0.49-0.93) |
| Not reported | 0.66 (0.61-0.72) | 0.57 (0.51-0.64) |

**Supplementary material 4: Subgroup analysis**




Supplementary figure 1. Funnel plot for functional outcome between patients with metformin use and patients without.




Supplementary figure 2. Funnel plot for survival status between patients with metformin use and patients without.

BMJ Open

# Effect of metformin on the clinical outcomes of stroke in patients with diabetes: a systematic review and meta-analysis

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|---|---|
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| Secondary Subject Heading: | Neurology |
| Keywords: | Stroke < NEUROLOGY, Meta-Analysis, Prognosis |
| | |

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# Tittle page

**Title:** Effect of metformin on the clinical outcomes of stroke in patients with diabetes: a systematic review and meta-analysis

**Keywords:** Stroke, Diabetes, Meta-analysis, Metformin, Clinical outcomes

**Authors and affiliations:** Jianyi Liu[a,b,1], Zhihua Huang[a,b,1], Fuqun Luo[a,b], Yizhi Guo[a,b], Yandeng Li[a,b], Jun Wen[a,b,*], Jianming Zhu[a,b,*]

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[1] First and second authors have same contributions.

# Effect of metformin on the clinical outcomes of stroke in patients with diabetes: a systematic review and meta-analysis


## Abstract

**Objectives** Stroke are major causes of death and disability globally, especially among diabetic patients. In this study, we aim to scrutinize the effects of metformin on the clinical outcomes of stroke in diabetic patients.

**Design** This study followed the PRISMA guidelines.

**Data sources** PubMed, Embase and Web of Science databases were searched between their inception and December 5, 2023.

**Eligibility criteria for selecting studies** Studies investigating the effect of metformin on the clinical outcomes of stroke in patients with diabetes were included.

**Data extraction and synthesis** The effect of metformin on the clinical outcomes of stroke in patients with diabetes was identified using combined odds ratios (ORs) and 95% confidence intervals (CIs).

**Results** A total of 11 studies involving 18525 participants were included in this review. Pooled analysis has been demonstrated that pre-stroke metformin use could reduce the probability of poor course after stroke by 34% in DM patients (OR =0.66, 95% CI: 0.61-0.72), and reduce the probability of death by 43% (OR = 0.57, 95% CI: 0.51-0.64).

**Conclusions** Pre-stroke metformin use is beneficial for the improvement of clinical outcomes in stroke patients with DM, although the potential bias should be carefully

considered.

**Keywords**: Stroke, Diabetes, Meta-analysis, Metformin, Clinical outcomes

**Strengths and Limitations of this study**

- The effect of metformin on the clinical outcomes of stroke in patients with diabetes was identified using combined odds ratios (ORs) and 95% confidence intervals (CIs).
- This study was processed in accordance with the PRISMA guidelines and was prospectively registered on PROSPERO.
- Most of included studies were retrospective, which is likely to increase the risk of confirmation bias, making it difficult to confirm causality.
- The frequency and duration of metformin use may be influence on the results, but this information was not adequate for consideration in this study, because few included studies provided this information.

## Introduction

Stroke, ischemic or hemorrhagic, is one of the primary causes of mortality and morbidity in the world[1]. Globally, the annual number of strokes and deaths due to stroke increased substantially from 1990 to 2019, particularly among people older than 70 years[2]. An estimated 17.8 million adults in China had experienced a stroke in 2020, with 3.4 million experiencing their first-ever stroke and another 2.3 million dying as a result[3]. In Europe, the prevalence of stroke was 9.2%, and the incidence was 191.9 per 100000 person-years[4]. In the United States, stroke mortality trends increased by 0.5% annually from 2012 through 2020 based on the national mortality data[5]. Despite advances in therapy, the clinical outcome for patients with stroke is still unfavorable. A large prospective observational study showed that the 5-year mortality rate after stroke was 51.7%[6]. The in-hospital mortality was 1.9% for stroke inpatients, and the 12-month fatality rate was 8.6%[7]. In light of this, it is crucial to identify in advance neuroprotective agents which can reduce neurological severity and improve clinical outcomes in stroke.

Disorders of glucose metabolism, highly prevalent and growing worldwide, are well-recognized risk factors for stroke, including type 1 and 2 diabetes mellitus (DM) and pre-diabetes[8]. These disorders are very common among stroke patients: 28% have pre-diabetes and 25% to 45% have DM[9]. Additionally, an association between DM and increased mortality, length of hospital stay, poorer functional outcomes after stroke has also been demonstrated[10-13]. To decrease the disparity between stroke

patients with DM and without, many attentions, to date, were paid to the influence of anti-diabetic agents on the severity of stroke and acute-phase outcomes in DM patients. Metformin, the first-line anti-diabetic drug, improves energy metabolism, and reduces oxidative stress, leading to improved balance of survival and death signaling in neurons[14]. A meta-analysis included 21 studies with 1392809 patients demonstrated that metformin monotherapy is effective in reducing stroke risk, but combined administration of metformin with other antihyperglycemic agents have no significant effect on stroke prevention in DM[15]. Besides serving as the protective factors of stroke, metformin may also be related with the clinical outcomes of stroke. Animal experiments showed that metformin plays a neuroprotection role in stroke and improves clinical outcomes triggered by stroke[16, 17]. In recent years, clinical studies have examined the effects of metformin in stroke outcomes, with some evidence that metformin pretreatment is associated with less severe strokes, improved functional outcome and lower mortality[18, 19]. In contrast, several studies showed that metformin use is not associated with in-hospital mortality and 1-year prognosis in diabetic ICH patients[20, 21]. In the context of existing inconsistencies between studies, the benefits of pre-stroke metformin use for improving the clinical outcome of stroke are continuing controversial.

In order to obtain insight into the issue mentioned above, we, in this study, searched for relevant published studies and performed a meta-analysis to scrutinize the effects of metformin on stroke outcomes.

## Methods

This study was processed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines and was prospectively registered on PROSPERO (CRD42024496056).

## Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this study.

## Literature search strategy

We systematically searched PubMed, Embase and Web of Science databases for studies published from inception to December 5, 2023. The search strategy divided by each database is provided in Supplementary material 1. In addition to database searches, we hand-searched the reference sections of included studies in the full-text review and undertook forward and backward citation tracking to find further eligible studies. All search results were imported into Endnote (X9), with any duplicates removed.

## Eligibility criteria and study selection

The exposure of interest was the pre-stroke metformin use, and the primary outcome was clinical outcome of stroke. Studies meeting the following criteria were included in this study: (1) reported the effect of pre-stroke metformin use on the outcomes of patients with stroke; (2) included patients with diabetes; (3) the sample size was beyond 10; (4) the report was not a review, comment, case report or letter; (5) full-

text articles were available, with no limit to the type of study designs. We did not place limitations on its country of origin, nor did we limit the age or gender of the included patients.

**Data extraction**

Firstly, two authors independently performed a screening of articles by reviewing titles and abstracts. Secondly, the full text of potentially eligible articles was retrieved, and relevant articles were assessed based on inclusion criteria. Any discrepancy between two authors was resolved by consensus or by consulting a third author. The following data from included studies were extracted: first author, study title, publication year, country, study design, sample size, patient demographics (i.e., gender distribution and mean/median age), and clinical outcomes (functional outcome and death). Where articles reported the outcome on multiple timepoints, the longest follow-up one was selected. The functional outcome after stroke were graded using a modified Rankin Scale (mRS) score ranging from 0 (no symptoms) to 6 (death). The mRS score was used to classify functional outcome as good course (score of 0 to 2) or poor course (score of 3 to 6).

**Quality assessment**

The quality of each included study was assessed using an eight-items modified version of the Newcastle-Ottawa Scale (NOS) for observational studies[22]. This scale estimates the quality of each study through three perspectives: the selection of sample; the comparability of groups; the ascertainment of outcome (Details were displayed in

Supplementary material 2). Two authors independently scored each study on every item in the scale. The higher the score, the better the methodologic quality of the study.

**Statistical analysis**

All statistical analyses were performed using R software version 4.0.2, and a two-sided P value of 0.05 or less was considered statistically significant. Data were recorded as the number of events in metformin use and non-metformin use groups. The pooled odds ratio (OR) and 95% confidence interval (CI) was calculated. This study used $I^2$ statistics and Chi-square test to evaluate between-study heterogeneity, with $I^2>50\%$ or $p<0.10$, indicating obvious heterogeneity, a random-effects model was used to evaluate the pooled results; otherwise, the fixed-effects model was applied. Publication bias was visually assessed using funnel plots, and quantified by the Egger's test. Additionally, sensitivity analysis of the pooled ORs was conducted by omitting 1 study in each turn, to estimate the impact of an individual study on the pooled results. A series of subgroup analysis and meta-regression according to region, publication year, study design, the type of stroke, follow-up duration and sample size were performed to explore the potential source of heterogeneity, and the pooled ORs between subgroups were compared using the Chi-square test.

**Results**

**Study characteristics**

A flow chart describing the selection of articles identified, included and excluded,

with reasons, is presented in Fig. 1. The search in the databases resulted in 1913 non-duplicate articles, 1852 of which were excluded after the screening of the titles and abstracts. The full text of the remaining 52 articles were retrieved and reviewed. Finally, the data from 11 articles were included in this study[18-21, 23-29]. One study was included through manual review of reference lists[30]. Nine studies were retrospective cohort study, and three were prospective cohort study. Nine studies reported the functional outcome, and eight articles the survival status (whether the patients had died or not). The eligible articles involved a total of 18664 patients, 7386 of which were with pre-stroke metformin use. The articles enrolled patients from a diverse range of geographical locations and ethnical populations. Detailed information on the included studies is summarized and presented in Table 1.

Table 1. Characteristics of included studies

| Author | Year | Country | Sample Size | N (Metformin/ control) | Design | Age | Stroke type | Outcomes |
|---|---|---|---|---|---|---|---|---|
| Horsdal et.al | 2012 | Denmark | 3841 | 563/3278 | PCS | MET+: 71.2 (63-79) | Ischemic stroke | 30 days, 1 year |
| Kuwashiro et.al | 2012 | Japan | 241 | 19/222 | RCS | 71±10 | Ischemic stroke | 3 months |
| Mima et.al | 2016 | Japan | 355 | 77/278 | RCS | 70.1±10.6 | Ischemic stroke | Discharge |
| Wu et.al | 2016 | Multiple | 374 | 148/226 | RCS | 68 (60-76) | ICH | 90 days |
| Westphal et.al | 2020 | European | 1919 | 757/1162 | RCS | MET+: 71, MET-: 74 | Ischemic stroke | 3 months |
| Tu et.al | 2021 | China | 730 | 281/449 | RCS | 65 (56-72) | ICH | Discharge, 1 year |
| Akhtar et.al | 2022 | Qatar | 2157 | 1132/1025 | RCS | 54.5±13.1 | Ischemic stroke | Discharge, 90 days |
| Curro et.al | 2022 | Italy | 139 | 69/70 | PCS | NR | Ischemic stroke | 3 months |
| Kersten et.al | 2022 | Netherlands | 937 | 592/345 | RCS | MET+: 75 (10), MET-: 76 (11) | Ischemic stroke | 3 months |
| Tu et.al | 2022 | China | 7587 | 3593/3994 | PCS | 66 (57–73) | Stroke | Discharge, 1 year |
| Akiyama et.al | 2023 | Japan | 160 | 55/105 | RCS | MET+: 75, MET-: 73 | Ischemic stroke | Discharge |
| Jian et.al | 2023 | China | 224 | 94/130 | RCS | MET+: 64 (54–71), MET-: 65 (56–74) | Ischemic stroke | 90 days |

NR: Not reported; RCS: Retrospective cohort study; PCS: Prospective cohort study.

**Quality assessment**

Ten included studies scored seven or above on the NOS checklist (Supplementary material 2) while two study scored six. This indicates that all included studies were of at least moderate quality. All patients met inclusion criteria in specific region were consecutively recruited within a certain period, and were divided into two groups according to the metformin or non-metformin use prior stroke, ensuring the representativeness and comparability of groups. The ascertainment of metformin use clearly were described in six studies[18, 21, 26-28, 30]. All including studies had a longer than 3 months follow-up to determine the functional outcome or survival status of the patients except for two studies reported discharge outcome only[25, 28].

**Effect of pre-stroke metformin use on prognosis of stroke**

The effect of pre-stroke metformin use on the improvement of functional outcome after stroke was assessed in nine cohort studies. Fig 2 shows the comparison of functional outcome between patients with pre-stroke metformin use and patients without, with individual and pooled ORs with corresponding CIs. Individual ORs ranged from 0.49 (95% CI: 0.24-0.97) to 0.87 (95% CI: 0.72-1.05), and pooled analysis showed that pre-stroke metformin use could reduce the probability of poor course after stroke by 34% in DM patients (OR =0.66, 95% CI: 0.61-0.72, $P < 0.001$). The application of Chi-square test and $I^2$ statistic showed that no significant heterogeneity existed among studies (p = 0.06, $I^2$ = 47%), and a fixed-effects model

was applied.

Also, eight studies with a total of 15908 patients reported the difference in survival status between patients with or without pre-stroke metformin use. The comparison of survival status between patients with pre-stoke metformin use and without is presented in Fig 3, with individual and pooled ORs with corresponding CIs. Individual ORs ranged from 0.16 (95% CI: 0.02-1.33) to 0.73 (95% CI: 0.47-1.13), and the pooled analysis indicated a 43% reduction in the probability of death after stroke (OR = 0.57, 95% CI: 0.51-0.64, $P < 0.001$). There was also no significant heterogeneity among studies (p = 0.78, $I^2$ = 0%), and a fixed-effect model was performed.

**Subgroup and sensitivity analysis**

The results of subgroup analysis showed that there are no significant differences across from different subgroup except for region (Supplementary material 3). Meta-regression was performed for all the two outcomes (functional outcome and survival status). None of the subgroups were significant for studies reporting the association between metformin use and clinical outcomes after stroke; hence, a multivariable meta-regression was not attempted. The results of sensitivity analysis showed that the pooled OR was steady, and removing one study did not change the significance of pooled OR. For functional outcome, the pooled OR ranged from 0.63 (0.57-0.68) to 0.68 (0.62-0.74); and for survival status, the pooled OR ranged from 0.56 (0.50-0.63) to 0.59 (0.52-0.67). The details are listed in Supplementary material 4.

**Publication bias**

Publication bias in the included studies was assessed by using Egger's test and funnel plot. The Egger's test indicated that there was no evidence of publication bias for the assessment of the effect of metformin use on functional outcome (p = 0.503) and survival status (p = 0.608). Also, the funnel plots revealed evidence of symmetry for functional outcome and survival status (Supplementary material 5).

**Discussion**

Metformin is a cheap, widely available, safe, and first-line anti-diabetic drug. Recently, metformin has also been demonstrated to be effective in decreasing the risk of stroke in DM patients[15]. In this study, we summarized evidence from published studies for now through a meta-analysis to prove that DM patients with pre-stroke metformin use had a better functional outcome and lower probability of death after stroke compared to those without. Thus, metformin in DM patients may not only be beneficial for reducing the risk of stroke, but also for improving the clinical outcomes after stroke.

As is well-known, hyperglycemia on admission was related with poor outcomes in stroke patients[31, 32], likely mediated through increased risk of infection and cardiac complications[23, 26, 33]. Pre-stroke glycemic control, as HbA1c level on admission, is a useful way to improve clinical outcomes in DM patients with stroke[34]. Thus, one possible pathway for the protective effect of metformin on stroke is through lowing blood glucose in DM patients. Moreover, interestingly, accumulating evidence

showed that although there is no statistically relevant difference between admission glucose levels of metformin group and sulfonylureas group[25], preadmission use of sulfonylureas does not affect stroke severity and clinical outcome among DM patients admitted with stroke[30, 35]. It seems to support the hypothesis of metformin pre-conditioning resulted in benefits besides its hypoglycemic effects.

The effect of metformin on clinical outcome was at least partially driven by the lower stroke severity on admission. The severity on admission was also a known determinant of chronic clinical outcomes after stroke[36-38]. A study, included 1281 patients with stroke, reported that the NIHSS score on admission, reflected the severity of stroke, could strongly predict the functional outcome after stroke, and patients with a score of NIHSS ≥ 16 on admission have a higher probability of death or sever disability than those without[38]. Several studies have shown that pre-stroke metformin use may be related with reduced neurological severity in stroke[18, 25]. In a cohort study with a total of 1919 stroke patients, patients with metformin treatment prior to stroke showed less severe strokes demonstrated by a lower NIHSS on admission compared to the non-pretreated patient group[18]. Similarly, a study identified metformin as the only antidiabetic drug to represent a significantly favorable determinant of stroke severity[25]. These results support the view that metformin may be an active option for DM patients, not only because of its position as a DM treatment, but also because of its neuroprotective effects[28].

One possible mechanism of metformin-induced neuroprotective in stroke is related

with neuronal adenosine monophosphate-activated protein kinase (AMPK), an important mediator of cellular energy homeostasis, highly expressed in neurons and activated under low cellular energy conditions, e.g., cerebral ischemia[39, 40]. Studies have demonstrated that AMPK plays a protective role in brain[41, 42]. Evidences from animal experiment showed that metformin, in acute stroke patients with DM, could improve the neurological function and oxidative stress status by the AMPK/mTOR signaling pathway and oxidative stress[43, 44]. However, it is worth noting that the neuroprotective effects require chronic use of metformin. Acute metformin use exacerbated stroke damage, enhanced AMPK activation, and led to metabolic dysfunction. Conversely, chronic metformin use was neuroprotective, improved stroke-induced lactate generation, and ameliorated stroke-induced activation of AMPK[39]. Therefore, the timing and duration of metformin use in DM patients should be taking consideration to achieve neuroprotection. Tian et.al indicated that a pretreatment time window no less than 7 days was required for the neuroprotection of metformin against acute brain injury, and the time window cannot be reduced by increasing metformin dosage[44]. The cumulative dynamics of metformin dosage may be a key of the protective effects for stroke by metformin pretreatment. Additionally, chronic metformin use after stroke is also beneficial for clinical outcome by inhibiting the inflammatory response, such as reduced IL-6 levels, stimulating vascular endothelial growth factor expression and promoting angiogenesis[25, 28]. Thus, metformin was a potential target in therapeutic intervention of stroke[45].

**Strengths and limitations**

To the best of our knowledge, this meta-analysis of effects of pre-stroke metformin use on the clinical outcomes of stroke in DM represents the first and pooled analysis of available evidence on this issue with a large pooled sample size. Nevertheless, although the results in this study are believed to highly stable, some limitations are acknowledged. First, only English studies included in this study, and the quantity of studies included was limited. Second, most of included studies were retrospective, which is likely to increase the risk of confirmation bias, making it difficult to confirm causality. Third, the frequency and duration of metformin use may be influence on the results, but this information was not adequate for consideration in this study, because few included studies provided this information. Whether metformin continues to be used after stroke is also unclear. Fourth, the effects of other diabetes treatments, including insulin and thiazolidinediones, were not evaluated. Given that insulin action might influence stroke prognosis, this may introduce potential treatment bias. Additionally, while metformin is generally well-tolerated, it is important to consider any potential risks or adverse effects associated with its use, particularly in the pre-stroke setting. While the 34% reduction in poor functional outcomes is statistically significant, further analysis and potentially additional research are needed to determine its clinical significance. In future researches, these factors should be considered at length. Therefore, the results on this study should be interpreted with caution.

## Conclusion

In conclusion, pre-stroke metformin use is beneficial for the improvement of clinical outcomes in stroke patients with DM, although the potential bias should be carefully considered. Metformin, as a known safety profile, may provide an economical and accessible therapeutic option in DM patients to improve stroke outcomes. Future researches in a large, prospective, randomized controlled trial are warranted to further elucidate the mechanisms underlying these associations and to determine whether metformin use may improve the clinical outcomes after stroke in DM patients.

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**Contributors** Study design: JMZ and JW, Data gathering: YL, FL and YG, Analysis: JL, Interpreting the results: JL, ZH, YG, Drafting: JL, ZH, Critically revised: All authors. Guarantor is Jianming Zhu.

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**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** Not applicable.

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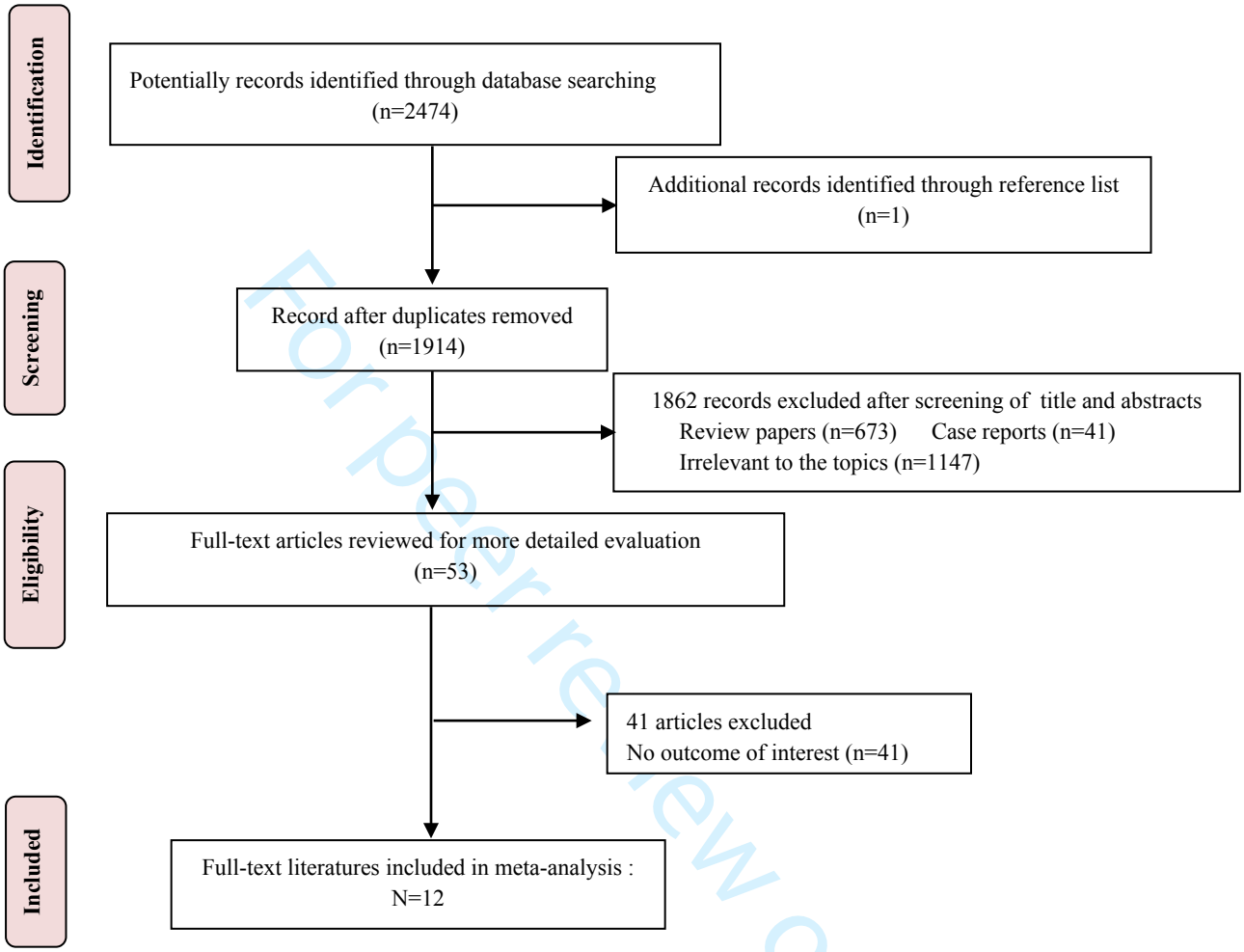
**Figure legends**

Fig. 1. Flow diagram to illustrate the study selection procedure.

Fig. 2. Forest plot for functional outcome between patients with metformin use and patients without.

Fig. 3. Forest plot for survival status between patients with metformin use and patients without.

Figure 1. Flow diagrom to illustrate the study selecton procedure.



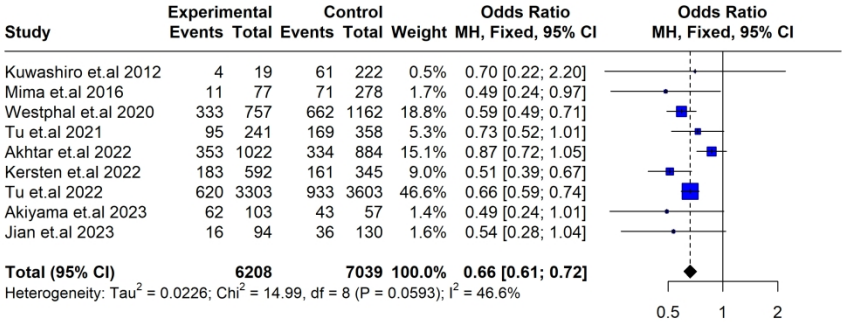



Fig. 2. Forest plot for functional outcome between patients with metformin use and patients without.

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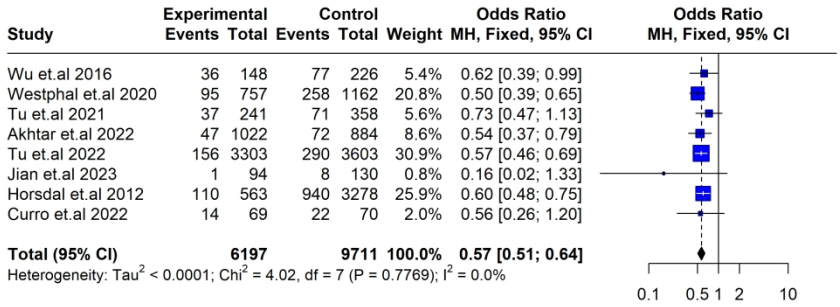



Fig. 3. Forest plot for survival status between patients with metformin use and patients without.

254x169mm (300 x 300 DPI)

**Supplementary material 1: Search terms**

**PubMed**

1. "Metformin"[mh] OR Metformin[tiab] OR Dimethylbiguanidine[tiab] OR Dimethylguanylguanidine[tiab] OR Glucophage[tiab] OR aron[tiab] OR diaformin[tiab] OR dmgg[tiab] OR glucohexal[tiab] OR glucophage[tiab] OR glucophage forte[tiab] OR glucophage retard[tiab] OR glucophage sr[tiab] OR glucophage xr[tiab] OR glucophage xr extended release[tiab] OR glucophagemite[tiab] OR gluformin[tiab] OR glumetza[tiab] OR glyciphage[tiab] OR glycon[tiab] OR i-max[tiab] OR islotin[tiab] OR la 6023[tiab] OR LA6023[tiab] OR metiguanide[tiab] OR neoform[tiab] OR nndg[tiab]
2. "Stroke"[mh] OR "Ischemic Stroke"[mh] OR "Embolic Stroke"[mh] OR "Cerebral Infarction"[mh] OR "Infarction, middle cerebral artery"[mh] OR "Brain infarction"[mh] OR "Stroke, Lacunar"[mh] OR "Thrombotic Stroke"[mh] OR Stroke[tiab] OR Cerebral Infarction[tiab] OR Brain infarction[tiab] OR middle cerebral artery infarct*[tiab] OR middle cerebral artery occlusion[tiab] OR Cerebral Infarct*[tiab] OR Brain Infarct*[tiab] OR Hemorrhagic Strokes[tiab] OR Stroke[tiab] OR Cerebrovascular Accident[tiab] OR Cerebrovascular Accident, [tiab] OR Apoplexy[tiab] OR Brain Vascular Accident*[tiab] OR Cryptogenic Embolism[tiab] OR Cerebral Infarct*[tiab] OR Subcortical Infarction[tiab] OR Choroidal Artery Infarction [tiab] OR MCA Infarction[tiab] OR Cerebral Artery Infarction[tiab] OR Cerebral Artery Embol*[tiab] OR Cerebral Artery Occlusion[tiab] OR Cerebral Artery Thromb*[tiab] OR Brain Venous Infarction[tiab] OR cerebral ischemia reperfusion injury[tiab] OR brain ischemi* reperfusion injury[tiab] OR brain ischemia/reperfusion[tiab] OR cerebral ischemia/reperfusion[tiab] OR cerebral reperfusion injury[tiab] OR reperfusion brain injury[tiab] OR acute cerebrovascular lesion[tiab] OR acute focal cerebral vasculopathy[tiab] OR brain vascular accident[tiab] OR cerebrovascular injury[tiab] OR cortical infarction[tiab] OR hemisphere infarct*[tiab] OR hemispheric infarct*[tiab] OR brain

stem infarction*[tiab] OR brainstem infarction[tiab] OR cerebellar infarction[tiab] OR brain ischemia[tiab] OR brain ischaemic attack[tiab] OR brain ischemic attack[tiab]

3. "Treatment outcome"[mh] OR "Treatment outcome"[tiab] OR "outcome"[tiab] OR "outcomes"[tiab] OR "prognosis"[mh] OR "prognosis"[tiab]

**Embase**

1. 'Metformin'/exp OR 'Metformin':ab,ti OR 'Dimethylbiguanidine':ab,ti OR 'Dimethylguanylguanidine':ab,ti OR 'Glucophage':ab,ti OR 'aron':ab,ti OR 'diaformin':ab,ti OR 'dmgg':ab,ti OR 'glucohexal':ab,ti OR 'glucophage':ab,ti OR 'glucophage forte':ab,ti OR 'glucophage retard':ab,ti OR 'glucophage sr':ab,ti OR 'glucophage xr':ab,ti OR 'glucophage xr extended release':ab,ti OR 'glucophage-mite':ab,ti OR 'gluformin':ab,ti OR 'glumetza':ab,ti OR 'glyciphage':ab,ti OR 'glycon':ab,ti OR 'i-max':ab,ti OR 'isotin':ab,ti OR 'la 6023':ab,ti OR 'la6023':ab,ti OR 'metiguanide':ab,ti OR 'neoform':ab,ti OR 'nndg':ab,ti

2. 'cerebral ischemia reperfusion injury'/exp OR 'cerebrovascular accident'/exp OR 'cardioembolic stroke'/exp OR 'brain infarction'/exp OR 'brain stem infarction'/exp OR 'cerebellum infarction'/exp OR 'brain ischemia'/exp OR 'transient ischemic attack'/exp OR 'Stroke':ab,ti OR 'Cerebral Infarction':ab,ti OR 'Brain infarction':ab,ti OR 'middle cerebral artery infarct*':ab,ti OR 'middle cerebral artery occlusion':ab,ti OR 'Cerebral Infarct*':ab,ti OR 'Brain Infarct*':ab,ti OR 'Hemorrhagic Strokes':ab,ti OR 'Stroke':ab,ti OR 'Cerebrovascular Accident':ab,ti OR 'Cerebrovascular Accident, ':ab,ti OR 'Apoplexy':ab,ti OR 'Brain Vascular Accident*':ab,ti OR 'Cryptogenic Embolism':ab,ti OR 'Cerebral Infarct*':ab,ti OR 'Subcortical Infarction':ab,ti OR 'Choroidal Artery Infarction ':ab,ti OR 'MCA Infarction':ab,ti OR 'Cerebral Artery

Infarction':ab,ti OR 'Cerebral Artery Embol*':ab,ti OR 'Cerebral Artery Occlusion':ab,ti OR 'Cerebral Artery Thromb*':ab,ti OR 'Brain Venous Infarction':ab,ti OR 'cerebral ischemia reperfusion injury':ab,ti OR 'brain ischemi* reperfusion injury':ab,ti OR 'brain ischemia/reperfusion':ab,ti OR 'cerebral ischemia/reperfusion':ab,ti OR 'cerebral reperfusion injury':ab,ti OR 'reperfusion brain injury':ab,ti OR 'acute cerebrovascular lesion':ab,ti OR 'acute focal cerebral vasculopathy':ab,ti OR 'brain vascular accident':ab,ti OR 'cerebrovascular injury':ab,ti OR 'cortical infarction':ab,ti OR 'hemisphere infarct*':ab,ti OR 'hemispheric infarct*':ab,ti OR 'brain stem infarction':ab,ti OR 'brainstem infarction':ab,ti OR 'cerebellar infarction':ab,ti OR 'brain ischemia':ab,ti OR 'brain ischaemic attack':ab,ti OR 'brain ischaemic attack':ab,ti

3. 'Treatment outcome'/exp OR 'Treatment outcome':ab,ti OR 'outcome':ab,ti OR 'outcomes':ab,ti OR 'prognosis'/exp OR 'prognosis':ab,ti

**Web of Science**

1. TS=( "Metformin" OR "Dimethylbiguanidine" OR " Dimethylguanylguanidine" OR " Glucophage" OR "aron" OR " diaformin" OR " dmgg" OR " glucohexal" OR " glucophage" OR " glucophage forte" OR " glucophage retard" OR " glucophage sr" OR " glucophage xr" OR " glucophage xr extended release" OR " glucophage-mite" OR " gluformin" OR " glumetza" OR " glyciphage" OR "glyc on" OR " i-max" OR " islotin" OR " la 6023" OR " la6023" OR " metiguanide" OR " neoform" OR " nndg" )

2. TS= ( "Stroke" OR " Cerebral Infarction" OR " Brain infarction" OR " middle cerebral artery infarct*" OR " middle cerebral artery occlusion" OR " Cerebral Infarct*" OR " Brain Infarct*" OR " Hemorrhagic Strokes" OR " Stroke" OR " Cerebrovascular Accident" OR " Cerebrovascular Accident, " OR " Apoplexy" OR " Brain Vascular Accident*" OR " Cryptogenic Embolism" OR " Cerebral Infarct*" OR " Subcortical Infarction" OR " Choroidal Artery Infarction " OR " MCA Infarction" OR " Cerebral Artery Infarction" OR " Cerebral Artery Embol*" OR " Cerebral Artery

Occlusion" OR " Cerebral Artery Thromb*" OR " Brain Venous Infarction" OR " cerebral ischemia reperfusion injury" OR " brain ischemi* reperfusion injury" OR " brain ischemia/reperfusion" OR " cerebral ischemia/reperfusion" OR " cerebral reperfusion injury" OR " reperfusion brain injury" OR " acute cerebrovascular lesion" OR " acute focal cerebral vasculopathy" OR " brain vascular accident" OR " cerebrovascular injury" OR " cortical infarction" OR " hemisphere infarct*" OR " hemispheric infarct*" OR " brain stem infarction*" OR " brainstem infarction" OR " cerebellar infarction" OR " brain ischemia" OR " brain ischaemic attack" OR " brain ischemic attack"

3. TS=("treatment outcome" OR "outcome" OR "outcomes" OR "prognosis")

## Supplementary material 2: Quality assessment of included studies

| Study | Item1 | Item2 | Item3 | Item4 | Item5 | Item6 | Item7 | Item8 | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Horsdal et.al 2012 | * | * | * | * | * | * | | | 7 |
| Kuwashiro et.al 2012 | * | * | * | * | * | * | | * | 8 |
| Mima et.al 2016 | * | * | | * | * | * | | * | 6 |
| Wu et.al 2016 | * | * | * | * | * | * | | * | 8 |
| Westphal et.al 2020 | * | * | * | * | * | * | | * | 8 |
| Tu et.al 2021 | * | * | | * | * | * | | * | 7 |
| Akhtar et.al 2022 | * | * | | * | * | * | | * | 7 |
| Curro et.al 2022 | * | * | | * | | * | | * | 6 |
| Kersten et.al 2022 | * | * | | * | * | * | | * | 7 |
| Tu et.al 2022 | * | * | | * | * | * | | * | 7 |
| Akiyama et.al 2023 | * | * | * | * | * | * | | * | 7 |
| Jian et.al 2023 | * | * | * | * | * | * | | * | 8 |

Items:

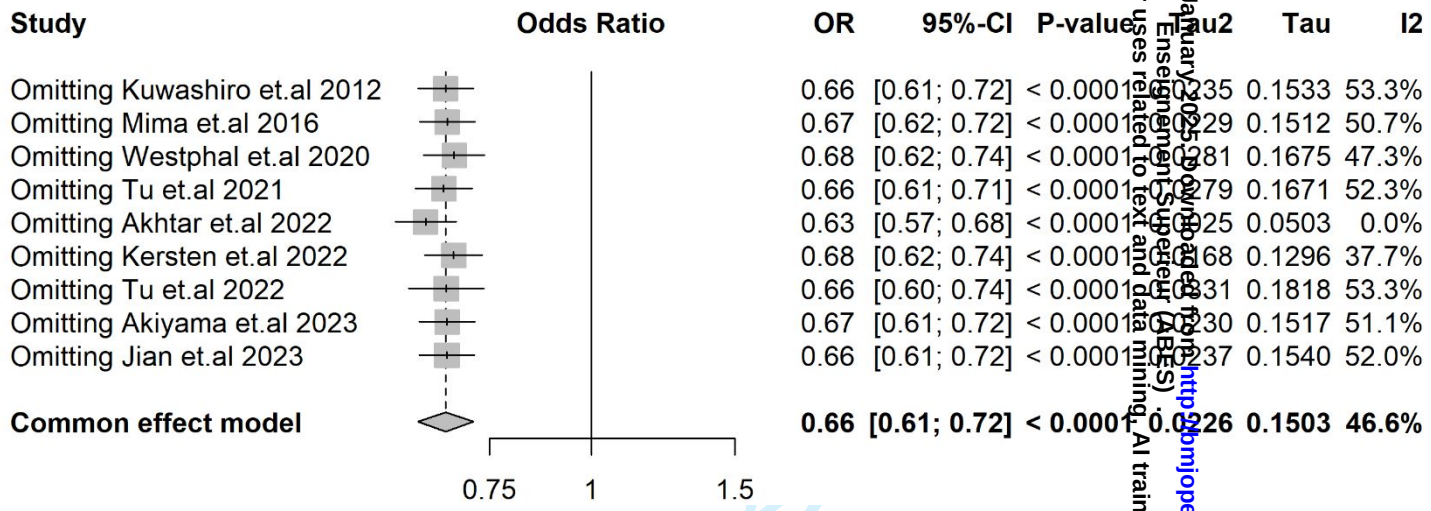
1. Was the exposure cohort representativeness?
2. Was the non-exposed cohort from the same population as the exposed cohort?
3. Was the ascertainment of exposure clearly defined, valid, reliable, and implemented?
4. Was the exposure of interest measured prior to the outcome(s) being measured?
5. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
6. Were the outcome measures clearly defined, valid, reliable, and implemented?
7. Was follow-up long enough for outcomes to occur?
8. Was loss to follow-up after baseline 20% or less?

**Supplementary material 3: Subgroup analysis**

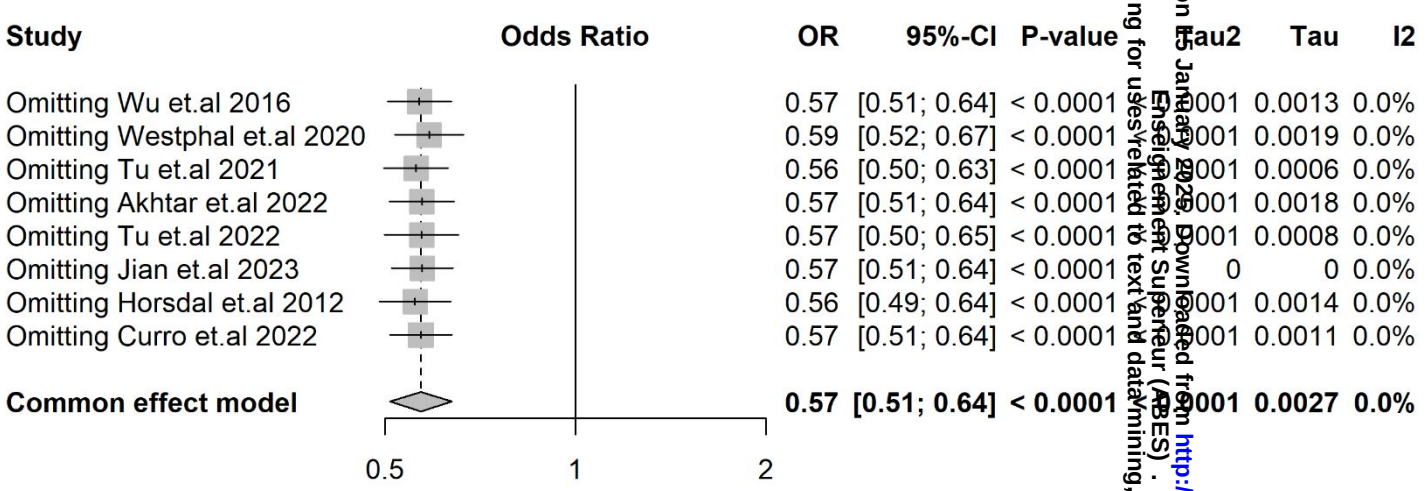
| Subgroup | No. of metformin /non-metformin | Functional outcome | $I^2$ | Test for subgroup differences | | No. of metformin/non-metformin | Survival status | $I^2$ | Test for subgroup differences | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $Chi^2$ | P | | | | $Chi^2$ | P |
| Public year | | | | 2.26 | 0.13 | | | | 0.02 | 0.88 |
| <=2020 | 853/1662 | 0.59 (0.49-0.70) | 0% | | | 1468/4666 | 0.57 (0.42-0.75) | 0% | | |
| >2020 | 5355/5377 | 0.68 (0.63-0.74) | 59.6% | | | 4660/4975 | 0.58 (0.49-0.68) | 0% | | |
| Sample size | | | | 2.92 | 0.09 | | | | 0.68 | 0.41 |
| <=1000 | 1126/1390 | 0.57 (0.48-0.69) | 0% | | | 483/714 | 0.65 (0.47-0.88) | 0% | | |
| >1000 | 5082/5649 | 0.68 (0.63-0.75) | 77.5% | | | 5645/8927 | 0.56 (0.50-0.63) | 0% | | |
| Study type | | | | 0.001 | 0.96 | | | | 0.22 | 0.64 |
| RCS | 2905/3436 | 0.66 (0.60-0.74) | 53.3% | | | 2262/2760 | 0.55 (0.46-0.66) | 0% | | |
| PCS | 3303/3603 | 0.66 (0.59-0.72) | NA | | | 3866/6881 | 0.58 (0.50-0.68) | 0% | | |
| Region | | | | 5.38 | 0.02 | | | | 0.20 | 0.90 |
| Asian | 4859/5532 | 0.70 (0.64-0.77) | 31.2% | | | 1320/4440 | 0.56 (0.47-0.66) | 11.3% | | |
| European | 1349/1507 | 0.57 (0.49-0.66) | 0% | | | 4660/4975 | 0.58 (0.49-0.68) | 0% | | |
| Follow-up duration | | | | 1.46 | 0.48 | | | | 1.18 | 0.28 |
| Discharged | 180/335 | 0.49 (0.30-0.81) | 0% | | | 0/0 | NA | NA | | |
| 3 months | 2484/2743 | 0.67 (0.60-0.75) | 69.8% | | | 2021/2402 | 0.52 (0.43-0.63) | 0% | | |
| 1 year | 3544/3961 | 0.67 (0.60-0.74) | 0% | | | 4107/7239 | 0.60 (0.52-0.69) | 0% | | |
| Disease type | | | | 0.33 | 0.85 | | | | 1.35 | 0.51 |
| IS | 2664/3078 | 0.66 (0.59-0.73) | 59.1% | | | 389/584 | 0.55 (0.47-0.64) | 0% | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ICH | 241/358 | 0.73 (0.52-1.01) | NA | 2436/5454 | 0.68 (0.49-0.93) | 0% |
| Not reported | 3303/3603 | 0.66 (0.61-0.72) | NA | 3303/3603 | 0.57 (0.51-0.64) | NA |

**Supplementary material 4: Sensitivity analysis**

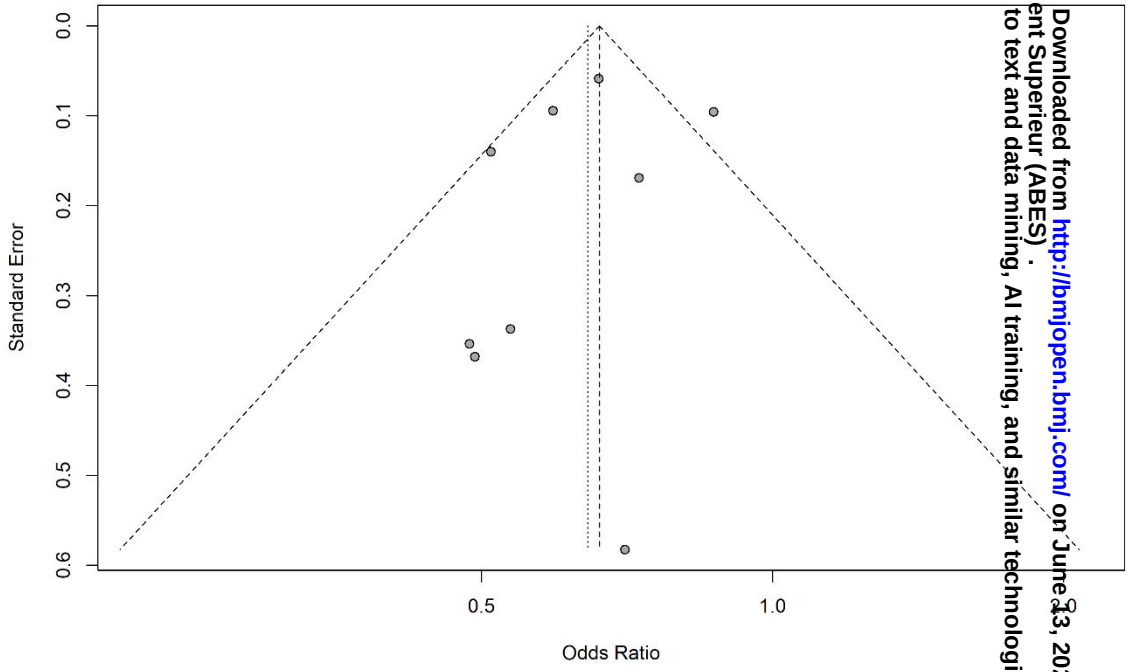



Supplementary figure 1. Sensitivity analysis for functional outcome between patients with metformin use and patients without.

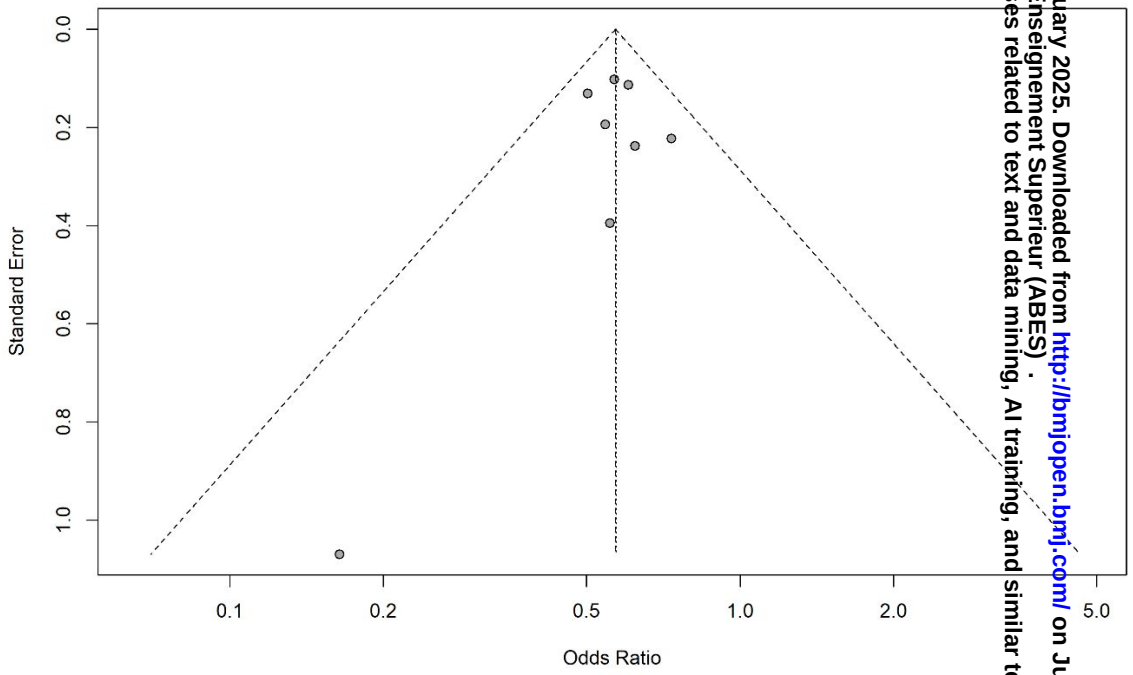



Supplementary figure 2. Sensitivity analysis for survival status between patients with metformin use and patients without.

**Supplementary material 5: Subgroup analysis**




Supplementary figure 3. Funnel plot for functional outcome between patients with metformin use and patients without.




Supplementary figure 4. Funnel plot for survival status between patients with metformin use and patients without.