Efficacy and Safety of Xingnaojing Injection for Emergency Treatment of Acute Ischemic Stroke: A Systematic Review and Meta-Analysis

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Background: Xingnaojing injection (XNJ) is derived from a traditional Chinese prescription named Angong Niuhuang pill. As an adjuvant treatment widely used in acute ischemic stroke (AIS), XNJ has proven to be effective with certain clinical evidence. The aim of this study is to collect the latest evidence and evaluate efficacy and safety of XNJ for emergency treatment of AIS.

Methods: We searched seven literature databases and two clinical trial registries from their inception to November 14, 2021 for randomized controlled trials (RCTs) examining the efficacy of XNJ for AIS. Two reviewers independently selected relevant trials, extracted data, and assessed the risk of bias. We pooled data into a meta-analysis using RevMan 5.4 software.

Results: Thirty-eight RCTs were included in this review, with a total of 3,677 participants. XNJ plus conventional treatments (CTs) showed a significant advantage, compared with CTs alone, in improving functional independence at 14 days ($RR = 1.70$, $95\% CI = 1.03$ to $2.81$, $p = 0.04$), neurological function ($MD_{NIHSS < 6h} = -3.81$, $95\% CI = -5.25$ to $-2.38$, $p < 0.00001$; $MD_{NIHSS < 24h} = -3.75$, $95\% CI = -4.92$ to $-2.59$, $p < 0.00001$; $MD_{NIHSS < 72h} = -3.74$, $95\% CI = -5.48$ to $-2.00$, $p < 0.0001$; $MD_{NIHSS < 14d} = -1.97$, $95\% CI = -3.25$ to $-0.69$, $p = 0.003$), and activities of daily living on the Barthel index ($MD_{BI-14day} = 9.97$, $95\% CI = 9.29$ to $10.65$, $p < 0.00001$; $MD_{BI-30day} = 10.04$, $95\% CI = 5.82$ to $14.26$, $p < 0.00001$). In addition, the results showed that XNJ plus CTs was superior to CTs alone in reducing IL-6, TNF-α, hs-CRP, and MMP-9. Regarding safety of XNJ, the incidence of adverse reactions in the XNJ group was lower than that in the control group ($RR = 0.57$, $95\% CI = 0.38$ to $0.87$, $p = 0.009$). The certainty of evidence was evaluated as low or very low for all.

Conclusion: XNJ appears to be effective and safe for emergency treatment of AIS. The first 72 h after the onset of stroke, in particular the first 6 hours, may be the optimum initiation time. However, further high-quality RCTs are warranted to determine an appropriate initiation time.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=233211], identifier [CRD42021233211].

Keywords: Xingnaojing injection, acute ischemic stroke, efficacy, safety, systematic review, meta-analysis
INTRODUCTION

Acute ischemic stroke (AIS) is a life-threatening medical condition that generally carries poor prognosis due to untimely treatment. There was a relative increase of roughly 8.9% in its global lifetime risk over the past 3 decades (Feigin et al., 2018). Stroke is the prominent cause of mortality and disability worldwide (Campbell and Khatri, 2020), and ischemic stroke accounts for about 80% (Donkor, 2018) of it, among which AIS is particularly dangerous. A Chinese large cohort study showed that the patients with AIS had a high rate of in-hospital recurrence of approximately 5.7% in the first 5 days, resulting in a higher in-hospital mortality rate (Yu et al., 2019). Its rate of death/disability within 3 months of onset in China was as high as 34.5–37.1% (Hao et al., 2011; Wang et al., 2013). To make matters worse, the incidence of stroke continues to increase with one in four people worldwide predicted to suffer from stroke during their lifetime (Collaborators, 2018). Therefore, AIS, bringing about a substantial economic and social burden, is of immense public health impact. Due to the demographic transitions, the public health burden is set to further rise over future decades (Adogu et al., 2015).

At present, the first-line emergency treatments for AIS recommended by the American Heart Association/American Stroke Association (AHA/ASA) are intravenous thrombolysis (IVT) and endovascular therapies (EVTs) (Powers et al., 2019). However, to date, both IVT and EVTs have low implementation rates due to the narrow therapeutic window of Angong Niuhuang pill, has been widely used already as an effective emergency treatment in the acute stage of cerebral infarction (Collaborators, 2018). Therefore, AIS, bringing about a substantial economic and social burden, is of immense public health impact. Due to the demographic transitions, the public health burden is set to further rise over future decades (Adogu et al., 2015).

At present, the first-line emergency treatments for AIS recommended by the American Heart Association/American Stroke Association (AHA/ASA) are intravenous thrombolysis (IVT) and endovascular therapies (EVTs) (Powers et al., 2019). However, to date, both IVT and EVTs have low implementation rates due to the narrow therapeutic window and hemorrhagic transformation (Tao et al., 2018; Hasnain et al., 2020; Sharobeam et al., 2021). Consequently, no other effective emergency therapies are available to address acute pathological reactions.

Angong Niuhuang pill is a first-aid Chinese patent medicine for acute stroke with more than 200 years of clinical application (Liu C et al., 2019). With the approval of the China Food and Drug Administration, Xingnaojing injection (XNJ), a derivative of Angong Niuhuang pill, has been widely used already as an emergency treatment in the acute stage of cerebral infarction (Liu et al., 2010). XNJ, extracted from Chinese botanical drugs via steam distillation, comprises Dryobalanops aromatica C.F.Gaertn [Dipterocarpaceae; Borneolum], Curcuma aromatica Salisb. [Zingiberaceae; Curcumae Radix], Gardenia jasminoides J. Ellis [Rubiaceae; Gardeniae Fructus], Moschus berezovskii Flerov, M. sifanicus Przewalski, or M. moschiferus Linnaeus [Cervidae; Moschus]. As for the process of preparation, 30 g of Curcumae Radix and 30 g of Gardeniae Fructus are first distilled with 1,500 ml of water, from which 1,000 ml of the distillate is collected; 7.5 g of Moschus and 250 ml of distilled water are then added to the abovementioned distillate, and 1,000 ml of the distillate is collected for standby; 1 g of Borneolum and 8 g of polysorbate 80 are ground and added to the distillate; finally, 8 g of sodium chloride is added, and the distillate is stirred, mixed, placed, and refrigerated overnight and then filtered, potted, and sterilized (Pharmacopoeia Committee of the Ministry of Public Health of the People’s Republic of China, 1998). With regard to the identified active components, borneol, whose concentration is traditionally used to control the quality of XNJ, should not be less than 0.7 g/L in accordance with the drug standards of the China Food and Drug Administration (China Food and Drug Administration, 2003). Moreover, by using gas chromatography–mass spectrometry (GC-MS), network pharmacology, and molecular docking technology, researchers recently found that the representative active components of XNJ also include geniposide, curdione, and muscone (Wu et al., 2021). Over the past few years, even though many studies have shown that the abovementioned components could inhibit oxidation, promote anti-inflammation, regulate the apoptosis, and activate autophagy (Liu et al., 2017; Lee et al., 2019; Fu et al., 2020; Zhang C et al., 2020), the complex mechanisms of this multiherbal preparation in cerebral infarction have been under exploration. Notably, in a rat model of middle cerebral artery occlusion–reperfusion, researchers have confirmed in vivo efficacy that XNJ could protect nerve cells and improve cerebral ischemia–reperfusion injury and conducted a preliminary investigation of the anti-inflammatory mechanisms, which probably relate to suppressing NLRP3 inflammasomes and enhancing SIRT1 expression (Zhang et al., 2018; Qu et al., 2019; Zhang et al., 2020). Furthermore, as for a therapeutically relevant dose range, an experiment has found that rats injected with 10 or 15 ml/kg of XNJ 24 h before ischemia and at the onset of reperfusion, respectively, showed greater improvement in neurological function and infarct volume with statistical significance than those injected with saline injection or 5 ml/kg of XNJ (Zhang et al., 2018). A systematic review of animal studies also supported these preclinical evidences (Ma et al., 2018). In the theory of traditional Chinese medicine (TCM), XNJ has effects of clearing heat and detoxification (TCM) of 53 trials published in 2017 concluded that XNJ might be a beneficial therapeutic method for cerebral infarction. However, lack of description regarding time to initiate XNJ and hemorrhagic transformation (Xu, 2010). Therefore, it can act on the critical pathological factors, heat toxin, and blood stagnation during the acute phase of ischemic stroke.

Continued evaluation of clinical efficacy from meta-analyses indicates that XNJ can benefit patients with ischemic stroke (Peng et al., 2014; Ma et al., 2017; Tian et al., 2021). A previous systematic review (Peng et al., 2014) of thirteen trials published in 2014 suggested that the efficacy and safety of XNJ in stroke patients were inconclusive, and it lacked subgroup analysis according to the types of strokes. The latest systematic review (Ma et al., 2017) of 53 trials published in 2017 concluded that XNJ might be a beneficial therapeutic method for cerebral infarction. However, lack of description regarding time to initiate XNJ has led to unclear efficacy evaluation of XNJ as a first-aid medicine in the acute phase. It was uncertain whether XNJ can be used immediately after the symptom onset. An overview of systematic reviews (Tian et al., 2021) pointed out that previous systematic reviews of XNJ for ischemic stroke had the following problems: 1) some critical items of AMSTAR2 were poorly reported, which included predefined protocol,
comprehensive search strategy, list of excluded studies, and reasons for exclusion. 2) Primary outcomes have always been measured using the total effective rate and neurological function instead of the modified Rankin Scale (mRS). 3) The clinical benefit in the acute phase of ischemic stroke is unclear.

To some extent, this review has sought to avoid the abovementioned limitations, providing relatively complete and up-to-date evidence on the use of XNJ for emergency treatment of AIS. The functional independence rate is used as the primary outcome. In addition, reporting of this review is in strict accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) 2020 statement (Page et al., 2021). Thus, this quantitative review aimed at answering the following questions: What is the additional clinical benefit of XNJ+conventional treatments (CTs) compared to CTs alone on AIS patients? What is the optimum initiation time of XNJ on AIS patients? To what extent is XNJ safe to be administered on AIS patients?

**METHODS**

**Protocol Register**

The protocol of this systematic review was prospectively registered in PROSPERO (Registration Number: CRD42021233211).

**Search Strategy**

We searched the following databases and registries from their inception to January 2021: PubMed, Cochrane Library, Embase, ClinicalTrials.gov, Chinese Biomedical Literature Service System (SinoMed), China National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), WanFang database, and Chinese Clinical Trial Register (ChiCTR). The detailed search strategies for all databases are presented in Supplementary Table S1.

On 14 November 2021, we updated the search using the same search strategies.

**Inclusion Criteria**

**Types of Studies**

We included randomized controlled trials (RCTs).

**Types of Participants**

We included participants of any age or gender with a primary clinical diagnosis of AIS.

**Types of Interventions**

The intervention group received XNJ combined with CTs, and the control group received the same CTs. Given the unclear overall scope of CTs, and in accordance with the AHA/ASA guidelines for early management of AIS (Powers et al., 2019) and the Chinese guidelines for diagnosis and treatments of AIS (Chinese Society of Neurology and Chinese Stroke Society, 2018), we decided to include trials using IVT, EVTs, antiplatelet treatment, statins, edaravone, or butylphthalide in consideration of the following factors:

1) In the Chinese guidelines, CTs are considered to also include anticoagulants, defibras, neuroprotective agents, and other cerebral circulation improving drugs. In the AHA/ASA guidelines, however, class of recommendation (COR) I only includes IVT, EVTs, and antiplatelet treatment. 2) For patients with AIS who qualify for statin treatment, in-hospital initiation or continuation of statin therapy is reasonable (COR IIa); 3) Edaravone (E Nomoto et al., 2019; Kobayashi et al., 2019) and dl-3-n-butylphthalide (NBP) (Wang et al., 2018; Xu et al., 2019) have extensive clinical foundation in China, and the latest established evidence indicated that they can not only improve the symptoms of ischemic stroke but also contribute to the long-term survival benefit.

**Types of Outcomes**

**Efficacy Outcomes**

The functional independence rate is used as the primary outcome. We defined functional independence as an mRS score of 0 to 2.

Secondary outcomes include neurologic deficit score (NDS), activities of daily living (ADL and Barthel Index), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), high-sensitivity C-reactive protein (hs-CRP), and matrix metalloproteinase-9 (MMP-9). There were three evaluation criteria for NDS – the National Institutes of Health Stroke Scale (NIHSS), the Chinese Stroke Scale (CSS), and the European Stroke Scale (ESS).

**Safety Outcomes**

Safety outcomes include incidence of adverse reactions and adverse events.

**Exclusion Criteria**

We excluded trials with the following features, in which: 1) other TCM treatments were applied in either the intervention or control group. 2) Outcomes were solely biochemical (for example, inflammatory markers) and not patient-centric (for example, the functional independence or NDS). 3) Outcomes were solely composite (for example, total effective rate) and not original (for example, NDS). 4) Outcomes were unclear (for example, NDS without definite evaluation criteria). 5) The course of treatment was less than 10 days or unclear. 6) The sample size was less than 60. 7) The language was not Chinese or English. 8) The full text was not available.

**Study Selection**

Two reviewers (LDW and XMF) independently performed literature selection according to the predefined eligibility criteria. The records retrieved in all databases were imported into NoteExpress 3.2, and the duplicate records were deleted. The records were first screened based on the title and abstract, and in cases of uncertainty, the full texts were obtained. Any disagreement between the paired reviewers was resolved by discussing with a third reviewer (WS).

**Data Extraction**

Two reviewers (LDW and YFC) independently extracted data from each trial using a predetermined data extraction form and then cross-checked data. Discrepancies were solved by discussion between the two reviewers or arbitrated by a third reviewer (WS).

We extracted the following data: 1) publication information (authors, country, and year of publication); 2) study designs...
methods of randomization, allocation concealment, and blinding); 3) participant baseline characteristics and sample size; 4) details of intervention and control groups; and 5) outcomes (dichotomous data were number of events and total subjects per group; continuous data were mean, standard deviation, and total subjects per group). In case of missing data or unclear information, we contacted the original authors to clarify the information.

## Risk of Bias Assessment

Two reviewers (LDW and XMF) independently assessed the risk of bias of the included trials. We used the Cochrane Risk of Bias Tool 2.0 (Sterne et al., 2019) to evaluate the following five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurements, and selective reporting. Each domain was judged as either “low risk of bias”, “some concerns”, or “high risk of bias”. If disagreements on the judgment were identified, a third reviewer (WS) was consulted.

## Data Analysis

Review Manager (RevMan 5.4) software was utilized to perform data analyses. Risk ratio (RR) was used for dichotomous data, while weighted mean difference (WMD) or standardized mean difference (SMD) was used for continuous variables, and all of which were demonstrated with effect size and 95% confidence intervals (CIs). A fixed-effects model was selected when no significant heterogeneity was identified (p ≥ 0.10, or I² ≤ 50%). Otherwise, a random-effects model was applied. We performed subgroup analyses based on the course of treatment and the initiation time of XNJ. When the heterogeneity was substantial (p < 0.10, or I² > 50%), sources of heterogeneity would be fully explored given the data were accurate. We further performed sensitivity analyses based on methodological quality and forest plots. After removing different trials in turn, we successively re-examined the meta-analysis results of the remaining trials to assess whether the statistical difference and heterogeneity change. If the findings changed evidently, the full texts of these trials would be checked, and we would interpret the results carefully.

To detect publication bias, we planned to generate funnel plots for meta-analyses including at least ten trials.

## Certainty Assessment

Two reviewers (LDW and YFC) independently assessed the certainty of the evidence using the Grading of
RESULTS

Study Selection
The search yielded 1961 records. There were 1234 duplicates, leaving 727 to be screened by title and abstract, from which 69 eligible records were retained for full-text evaluation. After careful evaluation, 30 reports were excluded. Ultimately, 38 trials met our inclusion criteria (Wu, 2009; Guan, 2011; Chen et al., 2012; Li et al., 2012; Tong and Zhu, 2012; Zhang et al., 2012; Dong and Fu, 2013; Qian and Jia, 2013; Luo, 2014; Wei and Cheng, 2014; Chen and Wu, 2015; Fang and Li, 2015; Lu et al., 2016; Wei et al., 2016; Zhang and Ai, 2016; Feng et al., 2017; Ji et al., 2017; Shen et al., 2017; Wang et al., 2017; Wang and Lu, 2017; Wu et al., 2017; Zhao, 2017; Fu, 2018; Guo, 2018; Lin et al., 2018; Ma, 2018; Xiao et al., 2018; Yin and Liu, 2018; Liu C et al., 2019; Zheng et al., 2019; Liang et al., 2020; Liu, 2020; Wu and Xu, 2020; Chen, 2021; He, 2021). Figure 1 shows details of trial selection. A list of 30 trials that appeared to meet the inclusion criteria but excluded is reported in Supplementary Table S2 along with citation and reasons for exclusion.

Study Characteristics
Thirty-eight RCTs involving 3,677 participants were included in this review, which included a total of 1841 patients in the intervention group and 1836 in the control group. Sample sizes of trials published from 2009 to 2021 ranged from 60 to 208. All trials were conducted in China and reported in Chinese. All interventions were XNJ in combination with CTs. Eight trials (Wei and Cheng, 2014; Zhao, 2017; Guo, 2018; Xiao et al., 2018; Zheng et al., 2019; Zhang, 2020; Shen, 2020; Chen, 2021; Dong et al., 2021) initiated XNJ treatment within 6 hours of stroke onset, and thirteen trials (Li et al., 2012; Zhang et al., 2012; Qian and Jia, 2013; Wei and Cheng, 2014; Liu et al., 2016; Zhang and Ai, 2016; Wang and Lu, 2017; Wu et al., 2017; Zhao, 2017; Fu, 2018; Guo, 2018; Lin et al., 2018; Ma, 2018; Xiao et al., 2018; Yin and Liu, 2018; Liu C et al., 2019; Zheng et al., 2019; Liang et al., 2020; Liu, 2020; Wu and Xu, 2020; Zhang, 2020; Zhao, 2020; Chen, 2021; Dong et al., 2021; He, 2021) initiated XNJ treatment within almost 1 day of stroke onset.

The proportion of functional independence at 14 days was reported by one trial (Ji et al., 2017). NDS was reported by all trials. Among them, 30 trials (Chen et al., 2012; Zhang et al., 2012; Qian and Jia, 2013; Wei and Cheng, 2014; Chen and Wu, 2015; Yang and Li, 2015; Lu et al., 2016; Zhang and Ai, 2016; Feng et al., 2017; Ji et al., 2017; Shen et al., 2017; Wang et al., 2017; Wang and Lu, 2017; Wu et al., 2017; Zhao, 2017; Guo, 2018; Lin et al., 2018; Ma, 2018; Xiao et al., 2018; Yin and Liu, 2018; Liu C et al., 2019; Zheng et al., 2019; Liang et al., 2020; Liu, 2020; Wu and Xu, 2020; Zhang, 2020; Zhao, 2020; Chen, 2021; Dong et al., 2021; He, 2021) adopted NIHSS, seven trials (Wu, 2009; Li et al., 2012; Tong and Zhu, 2012; Dong and Fu, 2013; Luo, 2014; Wei et al., 2016; Fu, 2018) adopted CSS, and four trials (Guan, 2011; Wei et al., 2016; Zheng et al., 2019; Liu, 2020) adopted ESS. The composition, source, and chemical characteristics of XNJ used in the included trials are presented in Supplementary Table S3. The other details are shown in Table 1.

Assessment of Risk of Bias
We summarized risk of bias of the included trials in Figure 2.

Domain 1: Risk of Bias Arising from the Randomization Process
For the generation of random sequence, 19 trials used a random number table (Zhang et al., 2012; Dong and Fu, 2013; Luo, 2014; Chen and Wu, 2015; Lu et al., 2016; Feng et al., 2017; Shen et al., 2017; Wang et al., 2017; Wang and Lu, 2017; Zhao, 2017; Fu, 2018; Guo, 2018; Lin et al., 2018; Liu H et al., 2019; Liang et al., 2020; Liu, 2020; Wu and Xu, 2020; Chen, 2021; He, 2021), one trial used a lottery (Dong et al., 2021), one trial used admission order (Yin and Liu, 2018), and the other seventeen trials lacked the adequate description of the randomization process (Wu, 2009; Guan, 2011; Chen et al., 2012; Li et al., 2012; Tong and Zhu, 2012; Qian and Jia, 2013; Wei and Cheng, 2014; Yang and Li, 2015; Wei et al., 2016; Zhang and Ai, 2016; Ji et al., 2017; Wu et al., 2017; Ma, 2018; Xiao et al., 2018; Zheng et al., 2019; Zhang, 2020; Zhao, 2020). No trial reported information regarding allocation concealment. Considering these, we judged 37 trials as “some concerns” and one trial (Yin and Liu, 2018) as “high risk of bias”.

Domain 2: Risk of Bias due to Deviations From the Intended Interventions
As no information of blinding was present in any of the included trials, we judged all trials as “some concerns” with doubt of deviations from the intended interventions.

Domain 3: Risk of Bias due to Missing Outcome Data
As all outcome data were available, we judged all trials as “low risk of bias” in this domain.

Domain 4: Risk of Bias in Measurement of the Outcome
As no information of blinding assessors was present, we judged all trials as “some concerns”.

Domain 5: Risk of Bias in Selection of the Reported Result
The planned outcome measurements and analyses in the Method section of published reports were fully reported without selection. Despite no available protocol, we judged all trials as “low risk of bias”.

In view of the abovementioned evaluation, we judged the overall bias of one trial (Yin and Liu, 2018) as “high risk of bias” and other trials as “some concerns”.

Efficacy Outcomes
Functional Independence Rate
One trial (Ji et al., 2017) determined the functional independence rate at 14 days after XNJ was initiated. The result demonstrated...
## TABLE 1 | Basic characteristics of the included trials.

| Study          | Sample size | Male/female | Age (year) | Course of disease/(day or hour) | Intervention group | Control group | Duration (day) | Outcomes |
|----------------|-------------|-------------|------------|---------------------------------|-------------------|---------------|----------------|----------|
|                | T C         | T C         | T C        |                                 | XNJ               | Combined with treatment |               | Efficacy | Safety |
| He, (2021)     | 47 47       | 24/23 25/22 | 58.24 ± 8.34 | 58.46 ± 6.79                  | (4–24) h          | (4–25) h       |                | CTs      | 30   | 2) 8) |
| Dong et al. (2021) | 62 62      | 32/30 33/29 | 58.59 ± 2.27 | 58.61 ± 2.31                  | (0–4.5) h         |                |                | CTs      | 14   | 2) 10| 11)  |
| Chen, (2021)   | 45 45       | 20/25 22/23 | 60.4 ± 6.8 | 59.6 ± 6.5                  | (2.4 ± 0.9) h     | (2.2 ± 0.8) h  |                | CTs      | 14   | 2)   |
| Wu and Xu, (2020) | 50 50      | 28/22 30/20 | 61.33 ± 9.81 | 60.86 ± 10.63                | (1.36 ± 0.61) d   | (1.45 ± 0.67) d |                | CTs      | 14   | 2) 5| 6) 7| 8) |
| Zhao, (2020)   | 47 47       | 23/24 23/23 | 62.79 ± 9.34 | 62.39 ± 10.43                | (0–14) d          |                |                | CTs      | 14   | 2) 10| 11)  |
| Zhang, (2020)  | 30 30       | 18/12 17/13 | 64.81 ± 6.94 | 64.89 ± 6.97                  | (3.45 ± 0.86) h   | (3.48 ± 0.85) h |                | CTs      | 14   | 2) 10| 11)  |
| Liu, (2020)    | 45 45       | 31/14 27/18 | 66.69 ± 6.98 | 67.42 ± 8.02                  | (0–24) h          |                |                | CTs      | 30   | 2) 4| 5) 10| 11) |
| Lian et al. (2020) | 43 43     | 29/14 27/16 | 59.49 ± 4.92 | 59.37 ± 5.28                  | (13.92 ± 2.65) h  | (13.85 ± 2.17) h  |                | CTs      | 14   | 2) 5| 10| 11) |
| Liu H et al. (2019) | 42 42     | 26/16 24/18 | 51.28 ± 6.14 | 51.69 ± 6.20                  | (0–72)            |                |                | CTs      | 14   | 2)   |
| Zheng et al. (2019) | 43 43     | 26/17 27/16 | 59.74 ± 7.97 | 60.12 ± 8.23                  | (3–4) h           |                |                | CTs      | 14   | 2) 4| 5) 11) |
| Yin and Liu, (2018) | 45 45     | 24/21 25/20 | 61.3 ± 12.70 | 60.8 ± 13.20                  | (23 ± 2.7) h      | (24 ± 2.2) h   |                | CTs      | 14   | 2)   |
| Ma, (2018)     | 43 43       | 19/24 21/22 | 57.76 ± 3.32 | 61.19 ± 3.71                  | (18.76 ± 3.32) h  | (19.46 ± 4.79) h |                | CTs      | 14   | 2) 10| 11)  |
| Xiao et al. (2018) | 70 70     | 48/22 47/23 | -           | -                               | (4.65 ± 0.19) h   | (4.49 ± 0.31) h |                | CTs      | 14   | 2) 6| 7) 8) 11) |
| Guo, (2018)    | 60 60       | 36/24 35/25 | 64.2 ± 12.63 | 63.3 ± 12.24                  | (4.4 ± 0.49) h    | (4.3 ± 0.58) h  |                | CTs      | 10   | 2) 8| 9)    |
| Fu, (2018)     | 37 37       | 25/12 23/14 | 65.36 ± 6.21 | 65.41 ± 6.17                  | -                 |                |                | CTs      | 14   | 3)   | 10| 11) |
| Lin et al. (2018) | 104 104    | 52/52 53/51 | 62.1 ± 5.3 | 61.3 ± 5.6                  | (0–72) h          |                |                | CTs      | 14   | 2) 10| 11) |
| Ji et al. (2017) | 63 63      | 30/33 35/28 | 62.4 ± 4.9 | 61.6 ± 4.5                  | (24–120) h        |                |                | CTs      | 14   | 1) 2| 5)    |
| Wang and Lu, (2017) | 49 49     | 27/22 26/23 | 67.41 ± 6.25 | 67.93 ± 6.14                  | -                 |                |                | CTs      | 14   | 2) 5| 6) 7| 10| 11) |
| Wu et al. (2017) | 50 50      | 26/24 25/25 | 63.1 ± 3.4 | 64.2 ± 3.6                  | (0–24) h          |                |                | CTs      | 30   | 2) 5| 11) |
| Feng et al. (2017) | 45 45     | 23/22 24/21 | 64.14 ± 7.24 | 64.72 ± 9.21                  | (0–72) h          |                |                | CTs      | 14   | 2) 5| 6) 7| 10| 11) |

(Continued on following page)
### TABLE 1 | Continued Basic characteristics of the included trials.

| Study                        | Sample size | Male/female | Age/(year) | Course of disease/ (day or hour) | Intervention group | Control group | Duration/ (day) | Outcomes |
|------------------------------|-------------|-------------|------------|----------------------------------|--------------------|---------------|----------------|----------|
|                             | T | C | T | C | T | C | T | C | XNJ | Combined with | treatment               | Efficacy | Safety |
| Wang et al. (2017)           | 35 | 35 | 18/17 | 19/16 | 60.2 ± 6.2 | 61.7 ± 5.3 | (18.1 ± 6.2) h | (16.5 ± 5.9) h | Xingnaojing injection | 30 ml/d | CTs | CTs | 14 | 2 (5) | 11 |
| Zhao, (2017)                 | 31 | 29 | 21/10 | 19/10 | 71.02 ± 7.31 | 70.52 ± 7.41 | (4.4 ± 0.49) h | (4.3 ± 0.58) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 (5) | 11 |
| Shen et al. (2017)           | 43 | 43 | 27/16 | 26/16 | 63.28 ± 6.48 | 62.54 ± 6.19 | (1–3) d | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 (5) | 10 (11) |
| Lu et al. (2016)             | 35 | 35 | 18/16 | 19/16 | 60.1 ± 8.5 | 59.9 ± 8.7 | (0–1) d | Xingnaojing injection | 30 ml/d | CTs | CTs | 14 | 2 | - |
| Zhang and Ai, (2016)         | 56 | 56 | 79/33 | -      | - | - | (23 ± 3) h | Xingnaojing injection | 30 ml/d | CTs | CTs | 14 | 3 (5) | - |
| Wei et al. (2016)            | 90 | 90 | 59/31 | 56/34 | 65 ± 5 | 64 ± 5 | (2.2 ± 0.8) h | (2.4 ± 0.7) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 21 | 2 (9) | - |
| Chen and Wu, (2015)          | 56 | 56 | 29/27 | 30/26 | 68.8 ± 7.3 | 68.3 ± 7.1 | - | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 | 11 |
| Yang and Li, (2015)          | 58 | 58 | 33/25 | 37/21 | 63.7 ± 6.5 | 62.0 ± 6.1 | (0–48) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 3 | 11 |
| Luo, (2014)                  | 60 | 60 | 35/25 | 38/22 | 60.2 ± 10.2 | 61.7 ± 11.2 | (38.12 ± 4.54) h | (41.58 ± 6.23) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 3 (5) | 11 |
| Wei and Cheng, (2014)        | 30 | 30 | - | - | - | - | (6–24) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 | 11 |
| Qian and Jia, (2013)         | 40 | 40 | 19/21 | 16/24 | 61.2 ± 5.9 | 61.6 ± 6.3 | (0–24) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 (6) | - |
| Dong and Fu, (2013)          | 35 | 35 | 21/14 | 19/14 | 58.74 ± 7.63 | 59.2 ± 7.95 | - | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 3 | - |
| Li et al. (2012)             | 30 | 30 | 15/15 | 13/18 | 62 | 63 | (0–24) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 3 | 11 |
| Zhang et al. (2012)          | 33 | 33 | 20/13 | 18/14 | 68.7 ± 10.5 | 69.2 ± 10.8 | (0–24) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 | - |
| Tong and Zhu, (2012)         | 64 | 66 | 38/26 | 40/26 | 61–85 | 62–84 | (0–72) h | Xingnaojing injection | 30 ml/d | CTs | CTs | 14 | 3 | 11 |
| Chen et al. (2012)           | 41 | 41 | 26/15 | 20/21 | 65.2 ± 12.6 | 63.5 ± 13.8 | - | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 2 | 11 |
| Guan, (2011)                 | 30 | 30 | 16/14 | 17/13 | 63.4 ± 10.7 | 61.4 ± 9.7 | - | Xingnaojing injection | 30 ml/d | CTs | CTs | 14 | 4 | - |
| Wu, (2009)                   | 54 | 52 | 30/24 | 30/22 | 63.45 ± 7.5 | 64.35 ± 7.36 | (0–72) h | Xingnaojing injection | 20 ml/d | CTs | CTs | 14 | 3 (5) | 6 (7) |

XNJ, Xingnaojing injection; CTs, conventional treatments; C, control group; T, intervention group; 1), Functional independence rate; 2), NIHSS; 3), CSS; 4), ESS; 5), ADL; 6), IL-6; 7), TNF-α; 8), Hs-CRP; 9), MMP-9; 10), Incidence of adverse reactions; 11), Adverse events.
that the 14-day functional independence rate of XNJ plus CTs was higher than that of CTs alone (RR = 1.70, 95% CI = 1.03 to 2.81, \( p = 0.04 \); Figure 3).

**NDS**

Thirty-eight trials reported change of NDS at multiple time points including 14 days (Wu, 2009; Guan, 2011; Chen et al., 2012; Li et al., 2012; Tong and Zhu, 2012; Zhang et al., 2012; Dong and Fu, 2013; Qian and Jia, 2013; Luo, 2014; Wei and Cheng, 2014; Yang and Li, 2015; Lu et al., 2016; Wei et al., 2016; Zhang and Ai, 2016; Feng et al., 2017; Ji et al., 2017; Shen et al., 2017; Wang et al., 2017; Wang and Lu, 2017; Zhao, 2017; Fu, 2018; Guo, 2018; Lin et al., 2018; Ma, 2018; Xiao et al., 2018; Liu H et al., 2019; Zheng et al., 2019; Liang et al., 2020; Wu and Xu, 2020; Zhang, 2020; Zhao, 2020; Chen, 2021; Dong et al., 2021), 21 days (Chen and Wu, 2015), 28 days (Yin and Liu, 2018), and 30 days (Wu et al., 2017; Liu, 2020; He, 2021). According to the different time points, a subgroup analysis was conducted under the same evaluation criterion of NDS.

**Scale 1: NIHSS**

In total, thirty trials assessed NDS using NIHSS. The statistical heterogeneity among them was substantial (\( I^2_{\text{NIHSS-14d}} = 97\% \), \( p < 0.00001 \); \( I^2_{\text{NIHSS-30d}} = 75\% \), \( p = 0.02 \)), so the random-effects model was used. As shown in the results, statistical difference was found between the two groups, which meant XNJ plus CTs was related to a more significant neurological recovery in NIHSS than CTs alone (\( \text{MD}_{\text{NIHSS-14d}} = -3.64, 95\% \text{ CI} = -4.30 \text{ to } -2.98, p < 0.00001 \); \( \text{MD}_{\text{NIHSS-21d}} = -4.49, 95\% \text{ CI} = -6.08 \text{ to } -2.90, p < 0.00001 \); \( \text{MD}_{\text{NIHSS-28d}} = -6.80, 95\% \text{ CI} = -7.55 \text{ to } -6.05, p < 0.00001 \); \( \text{MD}_{\text{NIHSS-30d}} = -2.52, 95\% \text{ CI} = -3.66 \text{ to } -1.38, p < 0.0001 \); Figure 4).

In the subgroup of the 14-day treatment course, we further performed a subgroup analysis according to the initiation time of XNJ. With the exception of two trials without specific course of disease (Chen et al., 2012; Wang et al., 2017), we divided 23 trials into four subgroups including within 6 hours (Zhao, 2017; Guo, 2018; Xiao et al., 2018; Zheng et al., 2019; Zhang, 2020; Dong et al., 2021) of stroke onset, 24 h (Zhang et al., 2012; Qian and Jia, 2013; Lu et al., 2016; Wei et al., 2016; Zhang and Ai, 2016; Wang and Lu, 2017; Ma, 2018; Liang et al., 2020; Chen, 2021), 72 h (Yang and Li, 2015; Feng et al., 2017; Shen et al., 2017; Lin et al., 2018; Liu H et al., 2019; Wu and Xu, 2020), and 14 days (Ji et al., 2017; Zhao, 2020). Despite the still significant heterogeneity (\( I^2_{\text{NIHSS<6h}} = 98\% \), \( p < 0.00001 \); \( I^2_{\text{NIHSS<24h}} = 94\% \), \( p < 0.00001 \); \( I^2_{\text{NIHSS<72h}} = 97\% \), \( p < 0.00001 \)), the results demonstrated that the effect size was largest for the 6-h subgroup and smallest for the 14-day subgroup (\( \text{MD}_{\text{NIHSS<6h}} = -3.81, 95\% \text{ CI} = -5.25 \text{ to } -2.38, p < 0.00001 \); \( \text{MD}_{\text{NIHSS<24h}} = -3.75, 95\% \text{ CI} = -4.92 \text{ to } -2.59, p < 0.00001 \); \( \text{MD}_{\text{NIHSS<72h}} = -3.74, 95\% \text{ CI} = -5.48 \text{ to } -2.00, p < 0.0001 \); \( \text{MD}_{\text{NIHSS<14d}} = -1.97, 95\% \text{ CI} = -3.25 \text{ to } -0.69, p = 0.003 \); Supplementary Figure S1). This suggested that the optimum initiation time of XNJ for AIS might be within 72 h, particularly the initial 6 hours. To reduce the heterogeneity, we conducted sensitivity analysis according to the distribution of trial confidence intervals on the forest plot. After removing trials whose confidence intervals hardly overlapped with those of the other trials, the heterogeneity was insignificant (\( I^2_{\text{NIHSS<6h}} = 45\% \), \( p = 0.16 \); \( I^2_{\text{NIHSS<24h}} = 14\% \), \( p = 0.32 \); \( I^2_{\text{NIHSS<72h}} = 0\% \), \( p = 0.89 \); Supplementary Figure S2). However, no obvious clinical heterogeneity was found in those removed trials.
A total of seven trials assessed NDS using CSS. The statistical heterogeneity was substantial ($I^2_{\text{CSS-14d}} = 80\%, p < 0.0001$), so we used the random-effects model. The results showed that CSS in the XNJ group was lower than that in the control group with statistical significance ($MD_{\text{CSS}} = -5.79, 95\% CI = -6.68$ to $-4.89, p < 0.00001$; Figure 4).

After a sensitivity analysis, we found that the exclusion of one trial (Wu, 2009) decreased heterogeneity by almost one-third ($I^2_{\text{CSS-14d}} = 57\%, p = 0.04$). We re-visited the full text and concluded that the major source of heterogeneity was likely to be the difference in CSS before treatment. The improvement of XNJ on neurological function might be closely associated to the severity of the disease. The trial was removed, and the remaining six trials were pooled again ($MD_{\text{CSS}} = -6.44, 95\% CI = -5.83$ to $-5.06, p < 0.00001$; Supplementary Figure S3).

We then performed a subgroup analysis based on the course of the disease. With the exception of two trials without a specific course of disease (Dong and Fu, 2013; Fu, 2018), we divided the remaining five trials into four subgroups including within 6 hours (Wei et al., 2016) of stroke onset, 24 h (Li et al., 2012), 48 h (Luo, 2014), and 72 h (Wu, 2009; Tong and Zhu, 2012). The results showed that the effect size of the 72-h subgroup was lower than those of the other subgroups with earlier XNJ initiated ($MD_{\text{CSS}} < 6\ h = -6.60, 95\% CI = -6.95$ to $-6.25, p < 0.00001$; $MD_{\text{CSS}} < 24\ h = -5.83, 95\% CI = -6.68$ to $-5.06, p < 0.00001$; $MD_{\text{CSS}} < 48\ h = -5.76, 95\% CI = -6.38$ to $-5.14, p = 0.002$; $MD_{\text{CSS}} < 72\ h = -5.62, 95\% CI = -6.28$ to $-5.06, p < 0.00001$; $MD_{\text{CSS}} < 144\ h = -5.59, 95\% CI = -6.23$ to $-5.06, p = 0.006$; Supplementary Figure S4). This correlation between the initiation time and efficacy is similar to that of NIHSS. However due to the small sample size, the optimal time of initiation requires further determination.

Scale 3: ESS

Four trials assessed NDS using ESS. Considering the substantial heterogeneity, we have applied the random-effects model for meta-analysis. The results showed that ESS in the XNJ group was higher than that of the control group with statistical significance ($MD_{\text{ESS-14d}} = 14.23, 95\% CI = 4.12$ to $24.34, p = 0.006$; $MD_{\text{ESS-30d}} = 14.14, 95\% CI = 5.71$ to $22.57, p = 0.001$; Figure 4).

To explore the sources of statistical heterogeneity, we revisited the original literature and found that the initial ESS before treatment of three trials was inversely proportional to the increase of ESS. This clinical heterogeneity may result in the correspondingly large statistical heterogeneity and weaken the reliability of the results.

### ADL–Barthel Score

Fourteen trials observed the ADL–Barthel score. Subgroup analysis was carried out according to the different observation time points. There was no significant heterogeneity ($I^2_{\text{BI-14d}} = 0\%, p = 0.95$; $I^2_{\text{BI-30d}} = 0\%, p = 0.35$), so the fixed-effects model was used. The pooled results showed that XNJ plus CTs was superior to CTs alone in improving ADL with a statistical difference ($MD_{\text{BI-14d}} = 9.97, 95\% CI = 9.29$ to $10.65, p < 0.00001$; $MD_{\text{BI-30d}} = 10.04, 95\% CI = 5.82$ to $14.26, p < 0.00001$; Figure 5).

### IL-6

Eight trials reported IL-6. We adopted SMD because of the large difference in the mean among trials (Wen and Li, 2007). The statistical heterogeneity was significant ($I^2 = 95\%, p < 0.00001$; Figure 6), and a random-effects model was used. Based on the use of SMD and large heterogeneity, we carried out descriptive analysis. All trials reported a statistical difference between the two groups, indicating that XNJ plus CTs is more effective than CTs alone in reducing IL-6.

Furthermore, a sensitivity analysis concluded that the differences in initial IL-6 may account for heterogeneity, and no other factors that could affect the heterogeneity were found. In the forest plot, three trials (Xiao et al., 2018; Wu and Xu, 2020; Zhao, 2020) had confidence intervals that hardly overlapped with those of the other five trials. We eliminated the three trials and pooled the remaining trials ($SMD_{\text{IL-6}} = -2.23, 95\% CI = -2.47$ to $-2.00, p < 0.00001$; Supplementary Figure S5). Heterogeneity among the five trials was insignificant ($I^2 = 25\%, p = 0.26$).

### TNF-α

Six trials reported TNF-α. We adopted SMD considering the large difference in the mean among trials. The statistical heterogeneity was substantial ($I^2 = 97\%, p < 0.00001$; Figure 7), and therefore a random-effects model was used. We carried out descriptive analysis. All trials reported a statistically significant difference between the two groups, which supported the effectiveness of XNJ in reducing TNF-α.

### Hs-CRP

Seven trials reported hs-CRP. The heterogeneity among trials was substantial ($I^2 = 98\%, p < 0.00001$; Figure 8), so a random-effects model was used. In addition to the baseline level of hs-CRP before treatment, no other factors that could cause such heterogeneity were found. We carried out descriptive analysis. All trials reported that XNJ plus CTs had a significant difference in reducing hs-CRP level compared with CTs alone.
MMP-9

Three trials reported MMP-9. The heterogeneity was moderate ($I^2 = 61\%, p = 0.08$), and a random-effects model was used. There was a consequential difference. XNJ plus CTs, as compared with CTs alone, was associated with more significant decrease in MMP-9 ($MD_{MMP-9} = -13.93, 95\% CI = -18.66 to -9.20, p < 0.00001; \textit{Figure 9}$).
After a sensitivity analysis, we found that excluding one trial (Guo, 2018) can significantly reduce heterogeneity. Treatment course and baseline level of MMP-9 were likely the major sources of heterogeneity. The trial was then removed, and the other two trials were pooled again ($MD_{MMP-9} = -11.69$, 95% CI $= -14.92$ to $-8.47$, $p < 0.00001$; Supplementary Figure S6). Heterogeneity between the remaining two trials was insignificant ($I^2 = 0\%$, $p = 0.85$).

### TABLE 1

| Study or Subgroup | XNJ+CTs Mean | SD | Total | CTs Mean | SD | Total | Weight | Mean Difference | IV, Fixed, 95% CI | Mean Difference | IV, Fixed, 95% CI |
|-------------------|--------------|----|--------|----------|----|--------|---------|----------------|-----------------|----------------|----------------|
| 1.2.1 ADL≤14d    | 51.46        | 20.12| 45  | 30.34    | 10.8| 45  |        | 0.7% | 12.11 [3.86, 20.36] |                      |                |                |
| Feng Jie 2017    | 74.91        | 18.29| 65  | 66.69    | 18.17| 61  |        | 1.2% | 8.32 [2.18, 14.46] |                      |                |                |
| Liang Jianjun 2020 | 72.39      | 10.48| 43  | 63.66    | 11.62| 43  |        | 2.0% | 8.73 [0.45, 16.13] |                      |                |                |
| Liu Changchun 2019 | 48.53        | 6.38| 42  | 39.22    | 6.02| 42  |        | 6.4% | 9.33 [6.68, 11.98] |                      |                |                |
| Liu Jiang 2014    | 64.45        | 9.36| 60  | 56.24    | 8.91| 60  |        | 4.2% | 8.21 [4.94, 11.48] |                      |                |                |
| Shen Li 2017      | 57.48        | 6.52| 43  | 48.32    | 5.36| 43  |        | 7.0% | 9.16 [6.64, 11.68] |                      |                |                |
| Wang Chengming 2017 | 48.34        | 1.32| 35  | 38.17    | 2.24| 35  |        | 60.4% | 10.26 [9.40, 11.12] |                      |                |                |
| Wang Chunchao 2017 | 48.51        | 6.37| 49  | 37.62    | 4.75| 49  |        | 9.1% | 10.69 [8.47, 12.91] |                      |                |                |
| Wu Qiancheng 2009 | 49.8        | 20.46| 54  | 51.45    | 21.48| 52  |        | 0.7% | 8.26 [0.27, 16.26] |                      |                |                |
| Wu Qinfeng 2020   | 68.17        | 9.25| 50  | 66.38    | 8.44| 50  |        | 3.7% | 8.79 [5.32, 12.26] |                      |                |                |
| Zhao Yichun 2017  | 84.05        | 15.02| 31  | 70.64    | 17.03| 29  |        | 0.7% | 13.41 [5.26, 21.56] |                      |                |                |
| Zheng Zhianguan 2019 | 79.82        | 14.01| 43  | 70.01    | 12.39| 43  |        | 1.4% | 9.81 [4.22, 15.40] |                      |                |                |

Subtotal (95% CI) 586 552 97.5% 9.97 [8.29, 10.65]

Heterogeneity: $Chi^2 = 4.68$, df = 11 ($P = 0.95$); $I^2 = 0$

Test for overall effect: $Z = 28.83$ ($P < 0.00001$)

1.2.3 ADL>14d

| Study or Subgroup | XNJ+CTs Mean | SD | Total | CTs Mean | SD | Total | Weight | Mean Difference | IV, Fixed, 95% CI | Mean Difference | IV, Fixed, 95% CI |
|-------------------|--------------|----|--------|----------|----|--------|---------|----------------|-----------------|----------------|----------------|
| Liu Cheng 2020    | 59.51        | 16.77| 45  | 47.24    | 13.44| 45  |        | 1.1% | 12.27 [6.00, 18.54] |                      |                |                |
| Wu Wanyu 2017     | 58.3        | 13.4| 50  | 50.1     | 15.6| 50  |        | 1.4% | 8.20 [2.50, 13.90] |                      |                |                |

Subtotal (95% CI) 95 95 2.5% 10.04 [5.82, 14.26]

Heterogeneity: $Chi^2 = 1.25$, df = 1 ($P = 0.99$); $I^2 = 0$

Test for overall effect: $Z = 4.57$ ($P < 0.00001$)

Total (95% CI) 655 647 100.0% 9.97 [8.31, 10.64]

Heterogeneity: $Chi^2 = 5.57$, df = 13 ($P = 0.96$); $I^2 = 0$

Test for overall effect: $Z = 29.20$ ($P < 0.00001$)

Test for subgroups differences: $Chi^2 = 0.03$, df = 1 ($P = 0.98$); $I^2 = 0$

### FIGURE 5

Forest plot of the ADL–Barthel score.

### FIGURE 6

Forest plot of IL-6.

### FIGURE 7

Forest plot of TNF-α.
Safety Outcomes

Incidence of Adverse Reactions

Nine trials reported the incidence of adverse reactions. Adverse reactions occurred in 31 out of 437 patients (7.1%) who received XNJ plus CTs and 54 out of 436 patients (12.4%) who received CTs alone. As the heterogeneity among the nine trials was insignificant ($I^2 = 0\%$, $p = 0.64$), we used a fixed-effects model. The results showed that XNJ plus CTs incurred fewer incidences of adverse reactions than CTs alone with a statistical difference ($RR = 0.57$, $95\% CI = 0.38$ to 0.87, $p = 0.009$; Figure 10).

Adverse Events

Twenty trials reported adverse events. Among them, nine studies (Li et al., 2012; Tong and Zhu, 2012; Luo, 2014; Wei and Cheng, 2014; Yang and Li, 2015; Wang and Lu, 2017; Wu et al., 2017; Zhao, 2017; Xiao et al., 2018) reported no serious adverse events in either group, and the other trials reported adverse events in both groups including gastrointestinal reactions, skin rashes, abnormal liver function, transient dizziness, slight headache, arrhythmia, dyspnea, gingival bleeding, and gastrointestinal bleeding. No participants discontinued the trial drug due to adverse events.

Publication Bias

The 14-day NIHSS of 24 trials was evaluated by the funnel plot, and the left–right asymmetry might be related to the Chinese publication of all included trials and unpublished negative results. As shown in Figure 11, the publication bias should be suspected to some extent.
Sensitivity Analysis
Sensitivity analysis was conducted for all outcomes except for the functional independence rate. We omitted trials one-by-one in order to observe the meta-analysis result of the remaining trials. None of these exclusions altered the statistical significance of results, which indicated the robustness of our results. However, we found significant change in heterogeneity of CSS, IL-6, and MMP-9.

GRADE Assessment
We used the GRADE approach to assess the quality of evidence for ten outcomes, and this ranged from “very low” to “low” with poor methodology, substantial heterogeneity, and publication bias. The certainty of evidence is summarized in Table 2.

DISCUSSION

Summary of Findings
In this review, we evaluated the efficacy and safety of XNJ as an emergency treatment for patients with AIS. We performed comprehensive literature search and identified 38 RCTs (3,677 participants). Compared with CTs alone, XNJ plus CTs was more effective for AIS in increasing the proportion of patients with independent function at 14 days (just one small sample trail), improving neurological function and restoring ability to perform daily activities.

Laboratory results showed positive effects of XNJ in improving IL-6, TNF-α, hs-CRP, and MMP-9. As a key cytokine in inflammatory response, IL-6 was found to be an independent risk predictor for AIS patients (Li et al., 2019), which may possibly be a new target in the prevention of short-term AIS death (Reiche et al., 2019). TNF-α, which plays an important role in the pathogenesis and the process of AIS, was also suggested to be a promising therapeutic target for the treatment of AIS (Wu et al., 2019). The anti-TNF therapy was found to be a feasible way to combat stroke disease via anti-inflammatory and metabolic mechanisms (Lin et al., 2021). Some studies (Huang et al., 2012; Vangilder et al., 2014) indicated that a high hs-CRP level was closely related to unfavorable long-term functional outcome and high rate of all-cause death 3 months after stroke. In addition, an elevated serum MMP-9 level in the acute phase of ischemic stroke was associated with increased risk of mortality and major disability, suggesting that serum MMP-9 could be an important prognostic factor for AIS (Zhong et al., 2017). Therefore, these outcomes are expected to be used as markers to predict the prognosis of AIS.

For safety evaluation, the results showed that the incidence of adverse reactions in the XNJ group was lower than that in the CT group. Nevertheless, it was worthy to note that both groups recorded slight gastrointestinal bleeding and arrhythmia. Although no difference in the incidence rate of these adverse reactions was observed between the two groups, it, nevertheless, suggested that close attention should be paid to the coagulation function and arrhythmia of patients after administering XNJ.

Limitations of the Included Trials
The trials we included had certain limitations. First, all participants were from China only, and no data were available from other countries. Second, with regard to interventions, we were unable to objectively evaluate the efficacy of XNJ combined with or without the first-line emergency treatments as few trials described the situation of IVT and EVT in patients. Third, with regard to outcomes, only one trial reported the proportion of functional independence, and the dependency was assessed only 2 weeks after the therapies were initiated. Thus, there was no sufficient evidence in improving the long-term function outcome.
In conducting this review, we have come across certain limitations that might undermine the conclusiveness of the findings. One of the major limitations was the substantial heterogeneity in NDS and biochemical outcomes, which affected the credibility of results. We speculated that the heterogeneity may have been resulted from the following factors:

1) Measurement of biochemical outcomes, with large fluctuation range, was easily influenced by different factors.
2) Clinical heterogeneity was present in the subtypes of ischemic stroke and the course of the disease.
3) Many trials failed to identify the syndrome of the subject, a concept of disease status in TCM.

Finally, and most importantly, key methodological issues were present, including poor randomization procedures, unblinded design, and lack of necessary follow-up. None of the included trials reported allocation concealment or blinding, and bias in selection, performance, and detection reduced the reliability of the findings.

**Limitations of the Review**

In conducting this review, we have come across certain limitations that might undermine the conclusiveness of the aforementioned findings. One of the major limitations was the substantial heterogeneity in NDS and biochemical outcomes, which affected the credibility of results. We speculated that the heterogeneity may have been resulted from the following factors:

1) Measurement of biochemical outcomes, with large fluctuation range, was easily influenced by different factors.
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3) Many trials failed to identify the syndrome of the subject, a concept of disease status in TCM.

When it comes to outcomes, most trials reported the total effective rate as a primary outcome, which we have chosen to exclude due to large uncertainty and non-standard evaluation of composite outcomes (Freemantle et al., 2003; Zhang Y Y et al., 2020). In spite of only one trial reporting the functional independence rate, we still used it as the primary outcome. The mRS has evolved as the primary outcome measure for acute stroke trials, and its application has demarcated effective and ineffective acute stroke therapies (Broderick et al., 2017; Powers, 2020). However, we excluded trials that used the entire ordinal distribution of the mRS as we contended that mRS measured by continuous statistical approach could affect the efficacy evaluation of overall functional independence due to a few severe cases. The incidences of death, hemorrhage, and other severe events should also be evaluated. In addition, the interventions included were different from the actual medical environment, which reduced the external validity.

**Implications for Future Research**

As a consequence of the abovementioned problems, we put forward the following suggestions to future researchers.

1) For the exploration of heterogeneity in TCM trials, proper recording of the subjects’ syndrome (证型) will allow better explanation of heterogeneity. In the TCM theory, syndrome is an indispensable part of “syndrome differentiation and treatment (辨证论治)”.
2) For the selection of primary outcomes, researchers are encouraged to assess good functional outcome using 90-day mRS in order to more accurately and intuitively measure clinical benefit. As accepted by the U.S. Food and Drug

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**TABLE 2 | GRADE summary of outcomes for XNJ+CTs versus CTs for patients with AIS.**

| Outcomes | No. of participants (studies) | Relative effect (95% CI) | Certainty of the evidence (GRADE) |
|----------|-----------------------------|--------------------------|---------------------------------|
| Functional independence rate (14d) | 126 (1) | RR 1.70 (1.05–2.81) | eCOO VERY LOWa,b,c,e |
| Incidence of adverse reactions | 873 (9) | RR 0.57 (0.38–0.87) | eCOO LOWa,b |
| NIHSS (14d) | 2,387 (25) | - | eCOO VERY LOWa,b,c,d,e |
| CSS (14d) | 739 (7) | - | eCOO VERY LOWa,b,c,d,e |
| ESS (14d) | 321 (3) | - | eCOO VERY LOWa,b,c,d,e |
| ADL–Barthel (14d) | 1,112 (12) | - | eCOO LOWa,b,c,d,e |
| IL-6 | 793 (8) | - | eCOO VERY LOWa,b,c,d,e |
| TNF-α | 620 (6) | - | eCOO VERY LOWa,b,c,d,e |
| hs-CRP | 731 (7) | - | eCOO VERY LOWa,b,c,d,e |
| MMP-9 | 330 (3) | - | eCOO VERY LOWa,b,c,d,e |

**Notes:**
- XNJ, Xingnaojing injection; CTs, conventional treatments; CI, confidence interval; RR, relative risks.
- Poor methodology including method of randomization and blinding.
- Publication bias.
- Only one study provided data.
- R² ≥ 50% for heterogeneity.
- Small number of RCTs, with small sample sizes.
Administration, the dichotomous approach in which mRS is divided into favorable and unfavorable outcomes should continue to be favored (Nunn et al., 2016). The length of follow-up should be at least 3 months (Lees et al., 2012). For phase II(b) trial, considering the time and resources required to measure mRS at 3 months and NIHSS within 1 week, a surrogate end point can also be used as a primary outcome endpoint (Chalos et al., 2020).

3) For the use of compound outcome the original NDS data should be provided when using the total effective rate to avoid exaggerating efficacy (McCoy, 2018), and only used as a secondary outcome.

4) For the applicability of results, it would be important to also consider the real-world studies. Our team has been conducting real-world studies of XNJ for acute stroke, hoping to provide helpful empirical data for clinical decision-making.

5) Safety takes precedence over efficacy when evaluating XNJ combined with EVTs or IVT for AIS. A mixed-methods research (Tian, 2021) suggested that early use of XNJ within 6 hours of AIS onset was associated with greater functional improvement. However, the unclear timing of XNJ initiation is still a prominent problem largely due to medical insurance restrictions and concerns around possible conflict between XNJ and IVT. Fortunately, some randomized, parallel-group, double-blind, multicenter trials are ongoing, such as a trial of the Tiantan hospital to evaluate efficacy and safety of XNJ for AIS patients with endovascular thrombectomy and a trial of the Dongzhimen hospital to investigate whether XNJ can be initiated after reperfusion therapy or when AIS is not eligible for IVT (Lai et al., 2017).

Future research should be designed based on rigorous methodology, including allocation concealment, blinding, appropriate sample sizes, and longer follow-up assessment. Trial protocol should be registered on the website in advance, and the results should be reported according to the guidelines of SPIRIT-TCM Extension 2018 (Dai et al., 2019) and CONSORT-CHM Formulas 2017 (Cheng et al., 2017). We will be monitoring closely and updating this systematic review over time as high-quality evidence emerges.

CONCLUSION

Initiating XNJ in the acute phase is effective in treating AIS. The optimum initiation time of XNJ for AIS might be the first 72 h after the onset of symptoms, in particular within the first 6 hours. However, due to insufficient evidence, it is inconclusive whether XNJ can be initiated immediately after the onset of AIS. However, due to the high risk of bias and substantial heterogeneity, the current evidence is not definitive. Given the low level of evidence, more rigorously designed and conducted RCTs, particularly those using the double-blind method, are warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

LW and WS contributed to study design. LW and XF drafted the manuscript. LW and XF retrieved literature, selected studies, and assessed studies. LW and YC extracted data, performed data analysis, and assessed evidence certainty. WS and XL proofread and revised the manuscript. YZ was responsible for the project administration and funding acquisition.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.839305/full#supplementary-material
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