Therapeutic effect of erythropoietin in patients with traumatic brain injury: a meta-analysis of randomized controlled trials

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OBJECTIVE Erythropoietin (EPO) exerts a neuroprotective effect in animal models of traumatic brain injury (TBI). However, its effectiveness in human patients with TBI is unclear. In this study, the authors conducted the first meta-analysis to assess the effectiveness and safety of EPO in patients with TBI.

METHODS In December 2015, a systematic search was performed of PubMed, Web of Science, MEDLINE, Embase, the Cochrane Library databases, and Google Scholar. Only English-language publications of randomized controlled trials (RCTs) using EPO in patients with TBI were selected for analysis. The assessed outcomes included mortality, favorable neurological outcome, hospital stay, and associated adverse effects. Continuous variables were presented as mean difference (MD) with a 95% confidence interval (CI). Dichotomous variables were presented as risk ratio (RR) or risk difference (RD) with a 95% CI. Statistical heterogeneity was examined using both I² and chi-square tests.

RESULTS Of the 346 studies identified in the search, 5 RCTs involving 915 patients met the inclusion criteria. The overall results demonstrated that EPO significantly reduced mortality (RR 0.69, 95% CI 0.49–0.96, p = 0.03) and shortened the hospitalization time (MD −7.59, 95% CI −9.71 to −5.46, p < 0.0001) for patients with TBI. Pooled results of favorable outcome (RR 1.00, 95% CI 0.88–1.15, p = 0.97) and deep vein thrombosis (DVT; RD 0.00, 95% CI −0.05 to 0.05, p = 1.00) did not show a significant difference.

CONCLUSIONS The authors suggested that EPO is beneficial for patients with TBI in terms of reducing mortality and shortening hospitalization time without increasing the risk of DVT. However, its effect on improving favorable neurological outcomes did not reach statistical significance. Therefore, more well-designed RCTs are necessary to ascertain the optimum dosage and time window of EPO treatment for patients with TBI.

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KEY WORDS traumatic brain injury; erythropoietin; favorable neurological outcome; mortality; deep vein thrombosis; meta-analysis
triguingly, EPO is also a multifunctional tissue-protecting agent that plays an important role in antiinflammatory, antiapoptotic, antioxidative, angiogenic, and neurotrophic effects. In addition, EPO exerts cytoprotective effects on endothelial cells, neurons, and glial cells. Several preclinical studies showed that EPO protects against neurological injury by decreasing secondary neuronal damage and thereby leading to improved neurological outcomes.

In 2007, a multicenter randomized controlled trial (RCT) revealed that EPO reduced the mortality of critical trauma patients. Numerous preclinical findings and clinical studies suggest that recombinant human EPO provides a neuroprotective effect that may be beneficial for patients suffering from stroke. Recently, concerns were raised over the effects of EPO in humans with TBI; however, several clinical trials using EPO as a treatment resulted in uncertain neuroprotectant efficacy. Nirula et al. conducted a clinical trial showing that EPO did not reduce neuronal cell death. A prospective study demonstrated that an erythropoiesis-stimulating agent provided a significant survival advantage in patients with severe TBI. More recently, in The Lancet, a large RCT demonstrated that EPO did not reduce the number of patients with severe neurological dysfunction or increase the incidence of deep vein thrombosis (DVT); however, the effect on mortality remained unclear. Therefore, our aim was to perform a meta-analysis of all RCT studies to determine the effect of EPO treatment on mortality, favorable outcome, hospitalization, and adverse events in patients with TBI.

**Methods**

This systematic review and meta-analysis was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Because all analyses were performed on previously published studies, ethical approval and patient consent were unnecessary. Two investigators (W.C.L. and L.W.) independently performed the literature search, data extraction, and quality assessment. Any disagreements were resolved by discussion.

**Literature Search and Selection Criteria**

In December 2015 we systematically searched PubMed, Medline, Web of Science, Embase, Google Scholar, and the Cochrane Library databases without restricting the date of publication. The following terms were searched: erythropoietin, EPO, head injury, head trauma, and traumatic brain injury. In addition, the reference lists of the identified articles and the “Related Articles” feature in PubMed were reviewed to maximize the probability of finding additional suitable papers. All English-language publications of RCTs that investigated the effect of EPO in patients with TBI were included, regardless of dose and duration of treatment.

The exclusion criteria were as follows: 1) nonrandomized clinical trial; 2) incomplete information for quantitative analysis (letters, review articles, and case reports); or 3) nonhuman models or non–English-language publications. Two reviewers independently screened and excluded papers based on the abstracts using the inclusion and exclusion criteria. Full-text articles of potentially relevant abstracts were retrieved and assessed independently according to an inclusion and exclusion checklist. All disagreements were resolved through discussion until a consensus was reached; if this failed, a third reviewer (X.F.Y.) was consulted.

**Data Extraction**

Two reviewers independently extracted data from the primary studies into a standard data extraction form. Data extracted from eligible studies included the first author, year of publication, study design, number of participants in each group, participant age and sex, treatment details, mortality and favorable outcome assessed by the Glasgow Outcome Scale (GOS) score, hospitalization time, and rates of DVT. The first effectiveness outcome was mortality. The second effectiveness outcome was favorable neurological outcome defined as the proportion of patients that achieved a GOS score of 4 or 5 and hospitalization time. For safety, we examined DVT rates.

**Quality Assessment**

The quality of the included studies was assessed by 2 independent reviewers. Any disagreement was resolved through discussion and consensus. Briefly, we used the Cochrane Collaboration’s tool to assess risk of bias according to the following domains: selection bias (random sequence generation and allocation concealment); attrition bias (incomplete outcome data); performance and detection bias (blinding of participants, personnel, and outcome assessment); reporting bias (selective reporting); and other bias (other sources of bias).

**Statistical Analysis**

Data synthesis and analysis were performed using RevMan software (version 5.3, Cochrane Collaboration). For continuous variables, results were calculated as the mean difference (MD) with a 95% confidence interval (CI). For dichotomous variables, we calculated the risk ratio (RR) or the risk difference (RD) with a 95% CI. Statistical heterogeneity was assessed using both the I² and chi-square tests. In cases in which I² was larger than 50%, a random-effects model was used, otherwise a fixed-effects model was used. Funnel plots were used to screen for potential publication bias.

**Results**

**Overview**

Our search strategy identified 346 articles, 328 of which were excluded by the title and abstract screening processes. Of the remaining 18 articles, full texts were accessed. Ultimately, 5 RCTs met our inclusion criteria and were included in this review. Fig. 1 shows a flow chart illustrating the above search process.

**Description of Studies**

The basic characteristics of the 5 studies are summarized in Table 1. The number of patients in the studies...
ranged from 16 to 603, and the total number of patients included in the meta-analysis was 915 (469 with EPO, 446 with placebo). The mean or median age of patients ranged from 25.2 to 46.5 years, and the majority were male. The EPO dosage ranged from 10,000 to 40,000 IU, and the first administration time was between 6 and 24 hours. The overall mortality rates ranged from 0% to 22.2%. The proportion of patients with a favorable functional outcome ranged from 29.8% to 55.6%. The rate of DVT occurrence ranged from 0% to 20%. In the study by Robertson and colleagues, 29 18.3% of the patients died in-hospital in the EPO group and none died in the placebo group. When the data were pooled, 11.1% of patients in the EPO group had died and 16.1% of patients in the placebo group had died. The results of the pooled analysis demonstrated that EPO significantly reduced mortality (RR 0.69, 95% CI 0.49–0.96, p = 0.03; Fig. 2). In addition, heterogeneity was not observed (I^2 = 0%; p = 0.83) in the overall analysis.

**Favorable Neurological Outcome**

Two studies 24,29 were included in the meta-analysis for the effect of EPO on favorable neurological outcome. In the study of Nichol et al., 24 55.6% of patients in the EPO group had a favorable neurological outcome compared with 55.1% of patients in the placebo group. Robertson et al. 29 showed that 37.0% of patients in the EPO group and 38.2% of patients in the placebo group had favorable neurological outcomes. The results of the pooled analysis demonstrated that EPO did not have a significant effect on favorable neurological outcome (RR 1.00, 95% CI 0.88–1.15, p = 0.97; Fig. 3). There was no heterogeneity among the studies (I^2 = 0%; p = 0.83). Two studies 1,2 showed that the mean GOS score and data were insufficient to be dichotomized into favorable and unfavorable outcomes. The study of Abrishamkar et al. assessed the neurological outcome by the MD and 95% CI; the results 1 revealed a higher GOS score in the EPO group compared with the placebo group (4.4 ± 0.3 vs 3.0 ± 0.3; p = 0.018). The same result 2 was also observed in the Aloizos et al. study, which showed that EPO may be associated with a higher mean GOS score (4.6 ± 0.5 vs 3.8 ± 0.6; p = 0.045).

**Hospital Stay**

There were only 2 studies 1,2 that provided data on hospital stay. In the Aloizos et al. study, 2 patients in the EPO group had a shorter stay than did patients in the placebo group (25.5 ± 3.3 vs 35.9 ± 11.6; p = 0.263). Abrishamkar and colleagues 1 showed that EPO treatment significantly decreased the length of stay in-hospital (20.4 ± 3.2 vs 27.5 ± 5.2; p = 0.02). When hospital stay data were pooled, the results demonstrated that EPO significantly reduced the length of stay in-hospital (MD −7.59, 95% CI −9.71 to −5.46; p < 0.00001; Fig. 4). In addition, there was no obvious heterogeneity (I^2 = 15%; p = 0.28) observed in the overall analysis.

**DVT**

Four of the studies 2,24,25,29 reported the rates of DVT...
### TABLE 1. Characteristics of the included studies

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Study Design</th>
<th>FU Duration</th>
<th>Diagnosis</th>
<th>No. w/ Tx/ Placebo</th>
<th>Intervention</th>
<th>Age (yrs)*</th>
<th>No. of Males (%)</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nichol et al., 2015</td>
<td>RCT</td>
<td>6 mos</td>
<td>Moderate or severe TBI</td>
<td>305/298</td>
<td>Epoetin alfa vs placebo, 40,000 IU, 1st dose w/in 24 hrs then w/kly for a max of 3 doses</td>
<td>30.5 (22.4–47.5)</td>
<td>30.5 (22.9–48.3)</td>
<td>256 (82.8)</td>
</tr>
<tr>
<td>Robertson et al., 2014</td>
<td>RCT</td>
<td>6 mos</td>
<td>TBI</td>
<td>102/98</td>
<td>Epoetin alfa vs placebo, 500 IU/kg, first dose w/in 6 hrs then w/kly for 2 more wks</td>
<td>31.5 (23–48)</td>
<td>29.0 (23-47)</td>
<td>88 (85.9)</td>
</tr>
<tr>
<td>Nirula et al., 2010</td>
<td>RCT</td>
<td>120 hrs</td>
<td>Moderate or severe TBI</td>
<td>11/5</td>
<td>Epoetin alfa vs placebo, 40,000 units intravenously administered w/in 6 hrs of time of injury</td>
<td>35 ±19</td>
<td>40 ± 26</td>
<td>8 (72.7)</td>
</tr>
<tr>
<td>Aloizos et al., 2015</td>
<td>RCT</td>
<td>6 mos</td>
<td>Severe TBI</td>
<td>24/18</td>
<td>Epoetin alfa vs placebo, 10,000 IU w/in 6 hrs then for 7 consecutive days</td>
<td>29.4 ± 1.3</td>
<td>46.5 ± 4.5</td>
<td>23 (95.8)</td>
</tr>
<tr>
<td>Abrishamkar et al., 2012</td>
<td>RCT</td>
<td>2 wks</td>
<td>Severe TBI w/ DAI</td>
<td>27/27</td>
<td>Epoetin alfa vs placebo, 2000 U rhEPO (w/in 7 hrs) for 6 doses in 2 wks (on days 2, 4, 6, 8, &amp; 10)</td>
<td>25.24 ± 5.4</td>
<td>27.3 ± 4.0</td>
<td>NR</td>
</tr>
</tbody>
</table>

DAI = diffuse axonal injury; DRS = Disability Rating Scale; FU = follow-up; ICP = intracranial pressure; ICU = intensive care unit; NR = not reported; NSE = neuron specific enolase; rhEPO = recombinant human EPO; Tx = treatment.

* Age was reported as median (range) in the studies of Nichol et al., 2015, and Robertson et al., 2014; age was reported as mean ± SD in the studies of Nirula et al., 2010, Aloizos et al., 2015, and Abrishamkar et al., 2012.
after the intervention. There was an incidence of 14.5% DVT in the EPO group and 14.8% in the placebo group. The pooled analysis revealed no significant difference in the occurrence of DVT between the EPO and the placebo groups (RD 0.00, 95% CI −0.05 to 0.05, p = 1.00; Fig. 5). There was little heterogeneity among the studies (I² = 45%; p = 0.14).

Sensitivity Analysis and Publication Bias

The results of the sensitivity analysis for favorable outcome, hospital stay, and DVT showed that none of the included studies alone had an obvious impact on the direction and magnitude of the outcomes. For overall mortality, when the studies by Nichol et al.24 or Robertson et al.29 were removed, the effect of EPO on mortality became insignificant (RR 0.71, 95% CI 0.41–1.24, p = 0.23; and RR 0.69, 95% CI 0.46–1.01, p = 0.06, respectively). However, we conducted a separate analysis of these 2 studies. The separate results of pooled analysis on mortality (RR 0.68, 95% CI 0.48–0.97, p = 0.04) were similar to the overall results of the 5 studies. Publication bias was evaluated by funnel plots. The shape of the funnel plot did not prove evidence of visible asymmetry (Fig. 6). Therefore, we concluded that our results are statistically consistent and robust.

Discussion

Recently published RCTs1,2,24,25,29 and meta-analyses27,28,36 have raised concerns about the efficacy and safety of EPO in patients with TBI. We present the first meta-analysis of the efficacy of EPO in treating patients with TBI. Our most important finding is that EPO treatment appears to be safe and effective in patients with TBI. EPO treatment was associated with a significant reduction (31%) in the risk of mortality. Furthermore, we found that EPO treatment shortened the length of stay in-hospital without increasing the incidence of DVT. However, its effect on improving favorable neurological outcome did not reach statistical significance.

The results of our meta-analysis are concordant with observations from patients with critical trauma. Corwin et
al.\textsuperscript{8} showed that treatment with EPO significantly reduced the 29-day mortality rate for critical trauma patients, including those with TBI. A meta-analysis by Turaga and colleagues\textsuperscript{36} reported that EPO could effectively decrease the number of units of blood transfused in patients with critical trauma.

Numerous clinical trials\textsuperscript{3,10,14,35,38} previously assessed the effect of EPO on neurological outcomes in patients after acute stroke. An RCT\textsuperscript{14} in 2002 showed that EPO was well tolerated in patients with acute ischemic stroke and associated with an improvement in clinical outcome. Recently, 3 RCTs\textsuperscript{3,35,38} demonstrated that EPO therapy significantly improved long-term neurological outcomes and decreased major adverse neurological events in patients after stroke. However, Ehrenreich et al. performed a randomized German multicenter EPO stroke trial\textsuperscript{10} and concluded that high-dose EPO (40,000 IU) therapy was associated with a significant increase in death and a nonsignificant increase in serious adverse events. According to these results, it appears that the dosage of EPO may be an important factor in its neuroprotective effect.

The present meta-analysis demonstrated that EPO lowered the mortality rate and shortened the hospitalization time for patients with TBI. Similar results were also found in a prospective\textsuperscript{33} and a retrospective study,\textsuperscript{34} which were not included in our study. We further analyzed the effects of EPO on vegetative state (defined as a GOS score of 2). The results of the pooled analysis demonstrated that EPO did not have a significant effect on vegetative state (RR 1.13, 95% CI 0.53–2.42, p = 0.75; Supplemental Fig. 1), which means that the decrease in mortality with EPO does not come at the expense of an increased incidence of vegetative state. Nevertheless, the concrete mechanism of mortality reduction in patients with TBI remains unclear. Previous preclinical studies\textsuperscript{15,30,36} reported that EPO not only has neuroprotective properties, but also supports regeneration. Patients with TBI commonly develop anemia, which is one potential cause of secondary injury and death. Therefore, EPO might reduce the mortality rate via a neuroprotective effect or simply by improving anemia or perhaps by modulating the acute inflammatory response.\textsuperscript{24} However, there is a need for further research focusing on the concrete mechanism. We found that EPO treatment shortened the hospital stays of patients with TBI; however, many important factors can affect the hospital stay of patients with TBI, such as age, severity of injury, functional independence, and medical complications. In addition, the length of hospital stay is less compelling than the neurological outcome or mortality. Therefore, caution is needed when interpreting these hospital stay findings.

Recently, Peng et al.\textsuperscript{27} performed a meta-analysis to assess the effect of EPO in experimental TBI and concluded that EPO might be beneficial in treating experimental TBI in terms of reducing lesion volume and improving neurobehavioral outcome. In our study, the pooled analysis of 2 studies\textsuperscript{24,29} that used a high EPO dosage (40,000 IU) demonstrated that EPO therapy had no effect on improving favorable neurological outcome. However, 2 other studies\textsuperscript{1} of low-dose EPO (10,000–12,000 IU) reported that it was associated with a higher mean GOS score (4.6 ± 0.5 vs 3.8 ± 0.6, p = 0.045; and 4.4 ± 0.3 vs 3.0 ± 0.3, p = 0.018) after treatment. Combined with previous research results in patients with stroke,\textsuperscript{10,14,35,38} these findings led us to conclude that EPO may be effective in improving favorable neurological outcome. However, more multicenter, large-sample, RCTs are required to determine the optimal dose of EPO for use in patients with TBI.

It is hypothesized that EPO increases arterial blood pressure, alters endothelial function, and may result in prothrombotic changes, which lead to a higher risk of throm-
Conclusions

Despite the limitations, our study demonstrated that EPO was effective in lowering the mortality rate and shortening hospital stay without increasing the incidence of DVT in patients with TBI. Our meta-analysis did not resolve existing uncertainties concerning the effectiveness of EPO in improving favorable neurological outcome. However, it does provide important information that can be used to guide future large multicenter RCTs.

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Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions
Conception and design: Yang. Liu. Acquisition of data: Yang, Liu, Wen, Xie. Analysis and interpretation of data: Liu, Wen, Xie. Drafting the article: Liu, Wang. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Yang. Statistical analysis: Liu, Wen. Study supervision: Yang.

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