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*Original Article*

# A multi-center clinical observation of recombinant human thrombopoietin for the treatment of sepsis-associated thrombocytopenia

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**Abstract:** Objective: To evaluate the clinical efficacy and safety of recombinant human thrombopoietin (rhTPO) in treating sepsis-associated thrombocytopenia. Methods: This is a randomized, parallel, controlled, multi-center study. 102 patients with sepsis and thrombocytopenia (peripheral platelet count, PLT ≤ $50\times10^9$/L) were randomly divided into rhTPO group and immunoglobulin (IVIG) group. Healthy volunteers were recruited as control. The primary outcome measure was platelet count within 9 days of intervention. Results: PLT increased gradually after treatment. On the 3rd day, 5th day, 7th day and 9th day of treatment, PLT was higher in rhTPO group than IVIG group ($p < 0.05$). Duration for PLT increasing to ≥ $50\times10^9$/L and ≥ $100\times10^9$/L was shorter in rhTPO group. On d2, d3, d5 and d7, plasma TPO level was higher in rhTPO group than intravenous human immunoglobulin (IVIG) group ($p < 0.01$). The total hospitalization cost (RMB) was lower in rhTPO group than IVIG group ($p = 0.041$). The transfusion amounts of platelets was less in rhTPO group than IVIG group ($p = 0.045$). Conclusion RhTPO promotes the recovery of platelet count without affecting platelet activation, and it reduces the amount of platelet transfusion and decreases medical costs in patients with sepsis-associated thrombocytopenia.

**Keywords:** Recombinant human thrombopoietin, sepsis and thrombocytopenia, platelet, multi-center

## Introduction

Sepsis is the leading cause of multiple organ dysfunction and non-cardiac death in ICU [1, 2]. Recent studies have reported that patients with serious infections usually have coagulation disorders. Thrombocytopenia is an important risk factor for the death of patients with sepsis [3]. Platelet transfusion has a quick effect. However, 10% to 30% of patients who need repeated platelet transfusions may develop allogeneic immune response [4]. Thrombopoietin (TPO) is a basic regulatory factor for the proliferation, differentiation, maturation of megakaryocytes, and the production of platelets. TPO plays the biological role through binding with its specific receptor Mpl, regulates the proliferation, differentiation and maturation of megakaryocytes, and the production of functional platelets [5]. Expressed by Chinese hamster ovary (CHO) cells, the recombinant human thromobopoietin (rhTPO) is purified and prepared to be the full-length glycosylated rhTPO (TPIAO, a product approved in China). It is effective for the treatment of chemotherapy-induced thrombocytopenia and idiopathic thrombocytopenic purpura in patients with solid tumors [6, 7]. Few studies on sepsis-associated thrombocytopenia have been reported [8]. This open, positive-controlled, randomized, multicenter study was designed to preliminarily evaluate the clinical efficacy and safety of rhTPO for the treatment of sepsis-associated thrombocytopenia.

## Methods

*Study design*

This is an open, positive-controlled, randomized, multi-center clinical study. The study was approved by the Ethics Committee of Tianjin First Central Hospital. The study group was the rhTPO group. The parallel control group was the intravenous human immunoglobulin group (IVIG group). 20 healthy volunteers were recruited as control (group C). The calculated effective cases were 80. In view of cases lost during follow-up and other confounding factors, 96 cases were planned to enroll. The subjects were randomized by 4 factors (gender, age, severity and platelet count) according to the allocation principle of minimized unbalanced index. Each sub-center was planned to recruit 16 cases (11 cases for rhTPO group; 5 cases for IVIG). The study was performed during September 2011 to September 2013.

*Case selection*

*The Inclusion criteria were:* patients with age 14 to 75 years; Patients who were diagnosed sepsis-associated thrombocytopenia according to American College of Chest Physicians and the Society of Critical Care Medicine (ACCP/SCCM) [9] diagnostic criteria: 1) Confirmed presence of bacteria or highly suspicious infectious foci; 2) 2 or more of the following signs: ① body temperature > 38°C or < 36°C; ② heart rate > 90 beats/min; ③ Respiratory rate > 20 times/min or partial pressure of arterial carbon dioxide ($PaCO_2$) < 32 mmHg; ④ Peripheral WBC count > $12.0\times10^9$/L or < $4.0\times10^9$/L, or immature granulocytes > 0.10; and patient with peripheral PLT ≤ $50\times10^9$/L, the attending physician or superior physician considered the patient needed treatment to elevate PLT [10].

*Patients were excluded if:* patients have malignant tumors; received immunomodulator therapy within 6 months; age < 14 years or > 75 years; Patients received cardiopulmonary resuscitation; Patients discharged or died within 24 h after admission; has end-stage liver or renal failure (Cr > 442 μmol/L); Thrombocytopenia caused by hematological system diseases; has serious cardiovascular and cerebrovascular diseases (New York Heart Association (NYHA) cardiac function IV, GCS score < 9 points); Patients with other diseases causing hypercoagulable state and thrombosis within 2 months; Massive hemorrhage (loss of 50% of total blood volume within 3 h, blood loss up to 150 ml/min, blood loss at the rate of 1.5 ml/(kg·min) within 20 min caused by severe trauma, major surgery and other reasons; Use of anti-platelet drugs, such as GPIIb/IIIa antagonist, clopidogrel, within 2 weeks before the treatment; Use of heparin anticoagulant in the first 2 weeks of medication.

*Drop-out/withdrawal criteria:* patients who did not meet the inclusion criteria or those who met the exclusion criteria after the review; Patients who asked to stop participating in the study or withdrew the consent; Violation of the clinical trial protocol; Withdrawal due to serious adverse events during the study; The investigators considered the patients were not suitable to continue to participate in the clinical study.

Apart from the withdrawal of subjects lost to follow-up, subjects who exited from the study due to other reasons should complete all the checks required at the last visit.

## Methods and procedures

All patients were observed on the basis of active infection control and the treatment of primary diseases. Patients in rhTPO group received TPIAO 300 U/kg/d, subcutaneous injected. TPIAO was stopped once PLT returned to $100\times10^9$/L or the absolute PLT increased to ≥ $50\times10^9$/L. TPIAO was used no longer than 5 days. Patients in IVIG group received intravenous infusion of human immunoglobulin 400 $mg\cdot kg^{-1}\cdot d^{-1}$ for 5 days.

*Indications of platelet transfusion [11]*

Prophylactic transfusion was performed if PLT < $10\times10^9$/L; PLT < $50\times10^9$/L before or during invasive procedures; Infusion therapy was performed if PLT < $50\times10^9$/L in acute active bleeding.

*Recorded indicators*

Disease severity was rated by acute physiology and chronic health evaluation II (APACHE II) scores within 24 h of admission; The major diseases and infectious pathogens were recorded in each subject: PLT, mean platelet volume (MPV), white blood cell count (Sysmex KX-21N blood cell analyzer, DKK-TOA Corporation,

Japan), DIC indicators [PT, APTT (automatic coagulation instrument, ACLTOP700, Wofen company, USA); D-dimer (automated fluorescence immunoassay analyzer, VIDAS, Merieux France)], hepatic function, renal function and other biochemical indicators (Beckman Coulter LX20; automatic biochemical analyzer, Beckman Coulter, Inc.), serum C reaction protein (CRP, Immage 800, BEKMAN, Germany) were detected before the treatment (d0), on the 1st day (d1, 24 h after the administration), the 2nd day (d2, 48 h after the administration), the 3rd day (d3, 72 h after the administration), the 5th day (d5, 120 h after the administration), the 7th day (d7, 168 h after the administration), and the 9th day (d9, 216 h after the administration). The transfusion of blood products, length of ICU stay, ICU mortality, hospital costs in ICU and 28-day mortality were recorded.

*Detection of platelet activation*

3 ml of blood was sampled in EDTA anti-coagulation tube (BD Vacutainer K2 EDTA 3.6 mg REF 367841) on d0, d1, d2, d3, d5, d7, d9 and centrifuged at 1000 g for 15 min. The supernatant was stored at -20°C refrigirater. The changes in the concentrations of plasma human solutable CD 40 ligand (sCD40L), human platelet factor 4 (PF4) and TPO were detected according to the instructions of ELISA kit. All antibodies were purchased from RayBiotech Company (RayBiotech, GA, USA).

*Data analysis*

SPSS 17.0 (IBM, Chicago, IL, USA) was used for statistical analysis. The categorical data were described with percentage and compared with $\chi^2$ test. The numerical data were presented as mean ± standard deviation ($\bar{x}$ ± S) and compared with independent sample t test. For repeatedly categorical data, mixed-effects model was applied using SAS 9.13. $p < 0.05$ was considered as statistically significant.

## Results

*Baseline data*

A total of 104 patients from 5 sub-centers were recruited. 1 subject in rhTPO group was excluded due to the application of immunomodulatory therapy. 1 subject in IVIG group asked for drug withdrawal and was dropped out. 102 patients completed the trials (see the flowchart).

63 cases including 39 males and 24 females were included into rhTPO group. 39 cases (24 males and 15 females) were included into IVIG group. The major diseases at admission were: pulmonary infection in 32 cases (including 18 cases of aspiration pneumonia, 14 cases of severe pneumonia), intestinal obstruction in 18 cases, gastrointestinal perforation/fistula in 10 cases, multiple trauma in 10 cases, acute pancreatitis in 8 cases, acute cholecystitis in 5 cases, 4 cases underwent surgery, acute drug poisoning in 4 cases, meningitis in 4 cases, acute suppurative appendicitis in 3 cases, postpartum uterine infection in 2 cases, and venous thrombosis of lower limbs in 2 cases. The main sites of infection occurred in lungs, abdomen, biliary tract, chest, urinary system, blood, limbs and skin. 25 patients have ≥ 3 sites of infection. All patients had positive results at least one of the etiological cultures (sputum, blood, catheter tip, drainage, or wound secretions, etc.). Gram-negative bacteria were mainly Escherichia coli in 18 cases, Acinetobacter bacilli in 15 cases, Pseudomonas aeruginosa in 15 cases, Stenotrophomonas maltophilia in 10 cases, Klebsiella pneumoniae in 8 cases, Enterobacter cloacae in 3 cases, Pseudomonas cepacia in 3 cases, and Enterobacter aerogenes in 3 cases. Gram-positive bacteria were mainly methicillin-resistant Staphylococcus aureus (MRSA) in 10 cases, Enterococcus in 7 cases, methicillin-resistant Staphylococcus epidermidis (MRSE) in 5 cases, hemolytic Staphylococcus in 5 cases, capitis Staphylococcus in 2 cases, and Enterococcus gallinarum in 2 cases. Fungi were mainly Candida albicans in 22 cases, Candida tropicalis in 15 cases, Candida Portugal in 3 cases, and Aspergillus in 3 cases. The differences in the constituent ratios of genders and diseases, APACHE II score, age distribution, platelet count and coagulation parameters before the treatment were not significant between the 2 groups of patients ($p > 0.05$). See **Table 1**.

*Platelet (PLT) parameters*

PLT before treatment was significantly lower in dead cases than survivals [(21.65 ± 7.49) $\times 10^9$/L vs. (27.14 ± 6.56)$\times 10^9$/L, $t = 3.448$, $p = 0.001$]. Compared with the healthy controls, PLT was significantly lower in patients with sepsis at enrollment [(34.32 ± 18.22 vs. 183.0 ± 40.0)$\times 10^9$/L, $p < 0.05$] (See **Table 2**) and gradually increased after treatment. The differences

**Table 1.** Comparison of the baseline characteristics at enrollment

| Parameters | rhTPO group | IVIG group | $t/c^2$ | *P* |
|---|---|---|---|---|
| Gender (male/female) | 39/24 | 24/15 | 0.001 | 0.970 |
| Age (y) | 57.2 ± 21.2 | 56.9 ± 18.3 | 0.064 | 0.949 |
| APACHE II (score) | 22.6 ± 6.1 | 23.0 ± 4.6 | 0.287 | 0.775 |
| PLT ($\times 10^9$/L) | 28.7 ± 9.7 | 27.5 ± 14.1 | 0.45 | 0.6533 |
| MPV (fL) | 11.12 ± 1.37 | 11.28 ± 1.22 | 0.56 | 0.5796 |
| sCD40L (ng/ml) | 7.12 ± 1.21 | 7.05 ± 2.07 | 1.48 | 0.1425 |
| PF4 (pg/ml) | 8.77 ± 3.38 | 8.56 ± 2.84 | 0.30 | 0.7464 |
| TPO (pg/ml) | 685.71 ± 261.92 | 721.06 ± 178.43 | 0.55 | 0.5832 |
| PT (s) | 18.2 ± 6.2 | 17.9 ± 5.7 | -0.29 | 0.7742 |
| APTT (s) | 54.48 ± 13.06 | 52.30 ± 17.19 | -0.17 | 0.8684 |
| INR | 1.53 ± 0.41 | 1.49 ± 0.54 | -0.34 | 0.7376 |
| WBC ($\times 10^9$/L) | 11.32 ± 8.44 | 12.76 ± 6.45 | -0.82 | 0.4132 |
| CRP (mg/L) | 134.89 ± 70.31 | 139.46 ± 73.88 | 0.16 | 0.8913 |
| RBC (×1012/L) | 3.17 ± 0.74 | 2.92 ± 0.53 | 1.48 | 0.1425 |
| HGB (g/L) | 97.20 ± 22.8 | 97.19 ± 13.3 | 0.66 | 0.5135 |
| ALT (U/L) | 113.79 ± 72.44 | 119.63 ± 68.37 | 0.28 | 0.8305 |
| AST (U/L) | 138.12 ± 79.57 | 141.65 ± 80.46 | 0.60 | 0.5872 |
| Cr (μmol/L) | 211.6 ± 104.64 | 199.18 ± 111.57 | 0.76 | 0.4110 |
| Primary disease | | | 3.978 | 0.680 |
| Lung infection | 22 | 10 | | |
| Intestinal obstruction | 11 | 7 | | |
| Gastrointestinal perforation/fistula | 4 | 6 | | |
| Acute Pancreatitis | 5 | 3 | | |
| Multiple trauma | 7 | 3 | | |
| Acute cholecystitis | 2 | 3 | | |
| Others | 12 | 7 | | |

Note: comparison of primary disease between rhTPO and IVIG groups was analyzed by $c^2$ test; Comparison of other numerical factors was done by student's t-test.

on d1 and d2 were not significant between the 2 groups ($p > 0.05$). PLT count was higher in rhTPO group than that in IVIG group on d3, d5, d7 and d9 ($p < 0.05$, **Figure 1A**). In rhTPO group, PLT on d3 was higher than that at enrollment (d0) and on d5 in IVIG group (all $p < 0.05$ *vs.* d0). The difference in PLT was not significant between rhTPO group and the healthy controls on d9, while PLT count in IVIG group was still lower than healthy controls ($p < 0.05$). In addition, the duration for PLT increasing to ≥ $50\times10^9$/L was shorter in rhTPO group *vs.* IVIG group [(3.79 ± 1.59) days vs. (4.63 ± 1.47) days, $t = -2.068$, $p = 0.043$]. Duration for PLT increasing to ≥ $100\times10^9$/L was shorter in rhTPO group compared with IVIG group [(6.31 ± 1.77) days vs. (8.83 ± 0.58) days, $t = -7.118$, $p < 0.001$].

Comparison of PLT after excluding subjects receiving platelet transfusion showed that PLT were lower in patients when compared with healthy controls at enrollment (d0). PLT gradually increased after treatment and was higher on d3 in rhTPO group. PLT elevated since d5 compared with baseline in IVIG group. PLT count was significantly higher in rhTPO group than IVIG group since d3. The difference in PLT was not significant between rhTPO group and healthy controls on d9. PLT count in IVIG group was still lower than rhTPO group on d9 (**Figure 1B**).

*MPV*

MPV was significantly larger in 102 subjects with sepsis-associated thrombocytopenia than healthy controls [(11.88 ± 1.27) fL vs. (9.64 ± 0.66) fL, $p < 0.001$)] (See **Table 2**) and did not change significantly at each time point in IVIG group. MPV increased on d3, d5 and d7 *vs.* enrollment in rhTPO group (d3:d1 $t = -2.01$, $p =$

**Table 2.** The baseline characteristics at enrollment

| Parameters | rhTPO group | IVIG group | Healthy controls |
|---|---|---|---|
| Gender (male/female) | 39/24 | 24/15 | 6/14 |
| Age (y) | 57.2 ± 21.2 | 56.9 ± 18.3 | 55±23.0 |
| APACHEII (score) | 22.6 ± 6.1 | 23.0 ± 4.6 | |
| PLT (×$10^9$/L) | 28.7 ± 9.7 | 27.5 ± 14.1 | 183.0 ± 40.0 |
| MPV (fL) | 11.12 ± 1.37 | 11.28 ± 1.22 | 9.64 ± 0.66 |
| sCD40L (ng/ml) | 7.12 ± 1.21 | 7.05 ± 2.07 | 3.19 ± 0.56 |
| PF4 (pg/ml) | 8.77 ± 3.38 | 8.56 ± 2.84 | 3.13 ± 0.82 |
| TPO (pg/ml) | 685.71 ± 261.92 | 721.06 ± 178.43 | 80.67 ± 5.16 |
| PT (s) | 18.2 ± 6.2 | 17.9±5.7 | 12.79 ± 0.99 |
| APTT (s) | 54.48 ± 13.06 | 52.30 ± 17.19 | 29.13 ± 2.82 |
| INR | 1.53 ± 0.41 | 1.49 ± 0.54 | 1.01 ± 0.11 |
| WBC (×$10^9$/L) | 11.32 ± 8.44 | 12.76 ± 6.45 | 7.06 ± 1.36 |
| CRP (mg/L) | 134.89 ± 70.31 | 139.46 ± 73.88 | 2.69 ± 0.99 |
| RBC (×1012/L) | 3.17 ± 0.74 | 2.92 ± 0.53 | 4.02 ± 0.42 |
| HGB (g/L) | 97.20 ± 22.8 | 97.19 ± 13.3 | 111.25 ± 10.80 |
| ALT (U/L) | 113.79 ± 72.44 | 119.63 ± 68.37 | 20.40 ± 7.25 |
| AST (U/L) | 138.12 ± 79.57 | 141.65 ± 80.46 | 22.55 ± 7.86 |
| Cr (μmol/L) | 211.6 ± 104.64 | 199.18 ± 111.57 | 60.04 ± 20.51 |
| Primary disease | | | |
| Lung infection | 22 | 10 | |
| Intestinal obstruction | 11 | 7 | |
| Gastrointestinal perforation/fistula | 4 | 6 | |
| Acute Pancreatitis | 5 | 3 | |
| Multiple trauma | 7 | 3 | |
| Acute cholecystitis | 2 | 3 | |
| Others | 12 | 7 | |

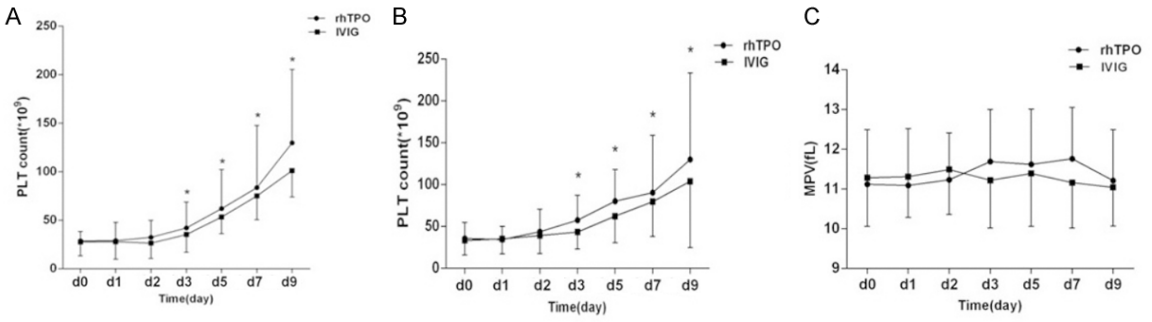


**Figure 1.** A. Comparison of platelet count between subjects with sepsis in rhTPO and IVIG groups. *$p < 0.05$, rhTPO group *vs.* IVIG group. B. Comparison of platelet count after excluding subjects receiving platelet transfusion; C. MPV was not different between rhTPO and IVIG groups. The statistical analysis was performed by student's t-test.

0.0472; d5:d1 $t$ = -2.11, $p$ = 0.0370; d7:d1 $t$ = -2.32, $p$ = 0.0219) and declined to baseline level ($t$ = 0.15, $p$ = 0.8776) on d9. There was no significant difference between rhTPO group and IVIG group (**Figure 1C**). The correlation between PLT and MPV was analyzed with Pearson correlation analysis. Overall: Pearson coefficient $r$ = 0.044, $p$ = 0.364. In rhTPO group, Pearson coefficient $r$ = 0.065, $p$ = 0.313. In IVIG group, Pearson coefficient $r$ = -0.083, $p$ = 0.268.

*Plasma sCD40L, PF4 and TPO levels*

Plasma levels of sCD40L and PF4 were higher in patients with sepsis-associated thrombocy-

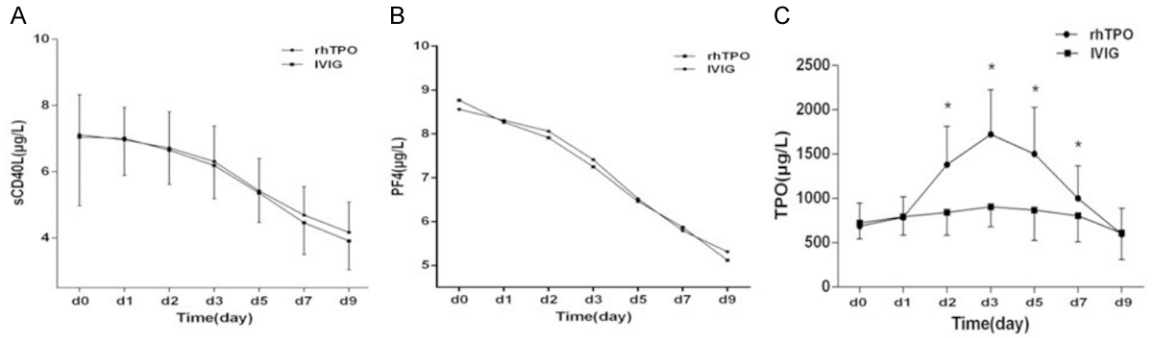


**Figure 2.** No significant difference was observed in (A) sCD40L; (B) PF4 between rhTPO and IVIG groups; (C) Plasma TPO level was higher in rhTPO group at d2, d3, d5, d7 than that in IVIG group, $p < 0.01$. The statistical analysis was performed by student's t-test.

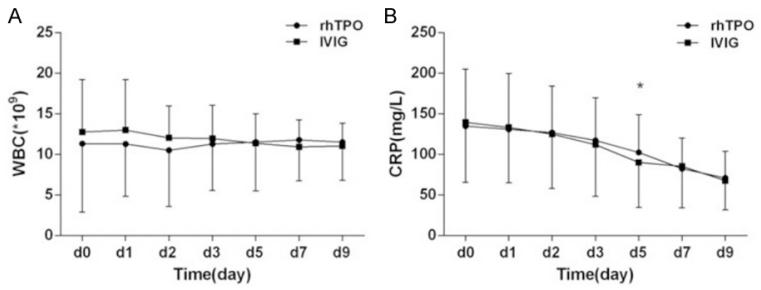


**Figure 3.** A. No significant difference was observed in WBC between rhTPO and IVIG groups; B. Serum CRP level was lower in IVIG group at d5 than rhTPO group, $p < 0.05$. The statistical analysis was performed by student's t-test.

topenia than healthy controls at enrollment [sCD40L: (7.03 ± 1.65) vs. (3.19 ± 0.56)] ng/ml, PF4: (8.29 ± 2.99) vs. (3.13 ± 0.82) ng/ml, ($p < 0.05$)] (See **Table 2**). Plasma sCD40L level was lower at d5, d7 and d9 than d0 in the 2 groups ($p < 0.05$). Plasma PF4 level was lower on d3, d5, d7 and d9 than that at baseline in the 2 groups ($p < 0.05$). Plasma levels of sCD40L and PF4 were higher than healthy controls ($p < 0.05$). The differences in sCD40L and PF4 were not significant between rhTPO group and IVIG group at each time point ($p > 0.05$) (**Figure 2A**, **2B**).

Plasma TPO level was higher in patients with sepsis-associated thrombocytopenia (peak level on d3) than healthy controls [d0: (789.35 ± 216.45) vs. control (80.67 ± 5.16) pg/ml), $p < 0.05$] (See **Table 2**). Plasma TPO elevated on d1, d2, d3, d5 and d7 than baseline in rhTPO group ($p < 0.05$). In IVIG group, plasma TPO level was elevated from d2 and declined on d7 to lower than baseline ($p < 0.05$). In rhTPO group, plasma TPO level was higher than that in IVIG group at d2, d3, d5 and d7 ($p < 0.01$), see **Figure 2C**.

*Blood WBC and CRP*

Blood WBC count and serum CRP level were higher in subjects with sepsis-associated thrombocytopenia than healthy controls [d0 (132.11 ± 68.34) vs. (2.69 ± 0.99) mg/L, $p < 0.05$)] (See **Table 2**). After the treatment, blood WBC count did not decline significantly. In rhTPO group, CRP level was lower on d3 to d9 than that at baseline ($p < 0.05$) and was lower from d2 to d9 than enrollment ($p < 0.05$) in IVIG group. The blood WBC count was not different between rhTPO group and IVIG group at each time point ($p > 0.05$). Serum CRP level was lower in IVIG group on d5 than rhTPO group ($p < 0.05$) (**Figure 3A**, **3B**).

*Coagulation indicators*

PT and APTT were significantly longer in subjects with sepsis-associated thrombocytopenia at enrollment than healthy controls ($p < 0.05$) (See **Table 2**). PT was shortened after the treatment in rhTPO and IVIG group ($p < 0.05$) but still longer than healthy controls ($p < 0.05$), except for d7 in rhTPO group. APTT shortened since d2 ($t = 3.42$, $p = 0.0009$) in rhTPO group compared with baseline ($p < 0.01$). APTT was shorter ($t = 2.32$, $p = 0.0223$) in IVIG group since d3 but still longer in the 2 groups than healthy controls

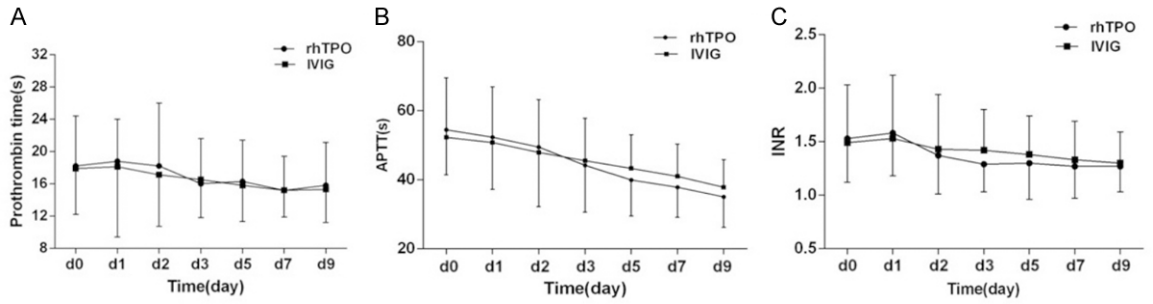


**Figure 4.** There is no significant difference in (A) PT; (B) APTT; and (C) INR between rhTPO and IVIG groups. The statistical analysis was performed by student's t-test.

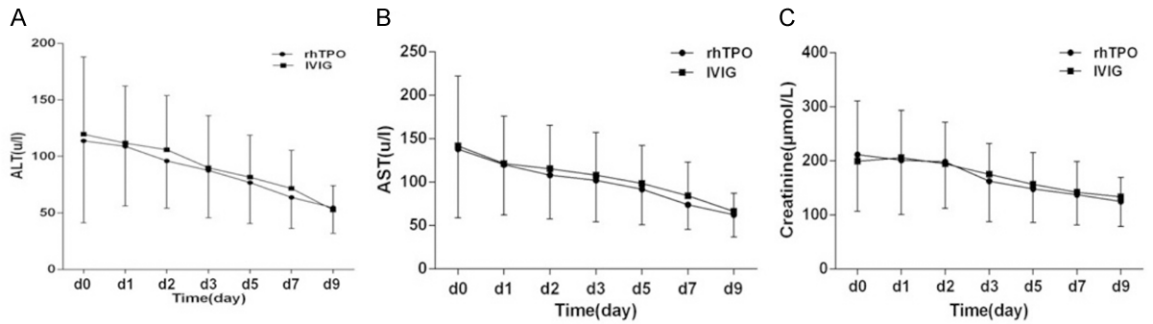


**Figure 5.** Comparison of (A) alanine transaminase (ALT); (B) aspartate aminotransferase (AST); (C) Cr between rhTPO and IVIG groups. The statistical analysis was performed by student's t-test.

except for d9 in rhTPO group. INR was significantly higher in subjects with sepsis-associated thrombocytopenia on d1 than healthy controls ($p < 0.05$) and was lowered from d2 to d9 than baseline ($p < 0.05$) (**Figure 4A-C**).

*Hepatic function and renal function indicators*

Blood ALT, AST and Cr levels were higher in the subjects with sepsis-associated thrombocytopenia compared with healthy controls at baseline ($p < 0.05$) (See **Table 2**). The hepatic function and renal function improved after treatment ($p < 0.05$ vs. at enrollment). No significant difference in hepatic function or renal function was observed between the 2 groups from d1 to d9 ($p > 0.05$) (**Figure 5A-C**).

*ICU stay, hospital costs in ICU and 28-day mortality*

The length of ICU stay has no difference between rhTPO and IVIG group [(14.9 ± 10.6) days vs. (16.4 ± 7.4) days, $t$ = -0.654, $p$ = 0.515]. The total hospitalization cost (RMB) was lower in rhTPO group than IVIG group [(141 900 ± 102 600) yuan vs. (186 900 ± 70 200) yuan, $t$ = -2.082, $p$ = 0.041]. The mortality rate during treatment has no significant difference between rhTPO and IVIG group [31.7% (20/63) vs. 35.8% (14/39), $\chi^2$ = 0.187, $p$ = 0.666]. The 28-day mortality has no difference between rhTPO and IVIG group [36.5% (23/63) vs. 41.0% (16/39), $\chi^2$ = 0.208, $p$ = 0.648](**Figure 6A-C**).

*Transfusion of blood products*

Among the 102 subjects, 38 patients (24/14) received platelet transfusion. 46 patients (28/18) received the transfusion of packed red blood cells. 37 patients (16/11) received plasma transfusion. The transfusion amounts of packed red blood cells and plasma were not significantly different between rhTPO and IVIG groups [(10.17 ± 10.39) U vs. (11.64 ± 8.41) U, $t$ = -0.519, $p$ = 0.606; (3515.67 ± 3884.05) mL vs. (4730.0 ± 4855.7) mL, $t$ = -0.721, $p$ = 0.287]. The transfusion amount of platelet was less in rhTPO group than IVIG group [(5.02 ± 6.10) U vs. (8.57 ± 7.33) U, $t$ = -2.043, $p$ = 0.045]. See **Figure 6D**.

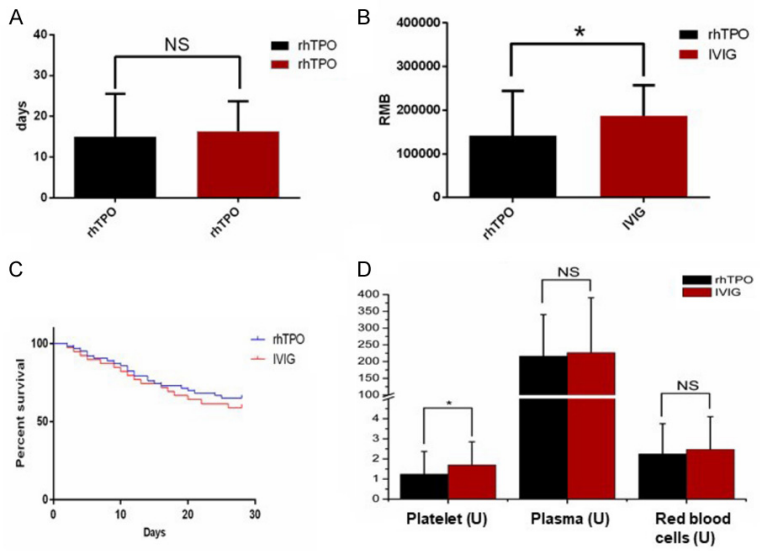


**Figure 6.** Comparison of (A) ICU stay; (B) hospital costs; a (C) 28-day mortality; and (D) transfusion of blood products between rhTPO and IVIG groups. The statistical analysis was performed by student's t-test.

## Discussions

Platelets are the key substance mediating blood coagulation and inflammatory reactions in sepsis. After binding with receptor of pathogens, the activated platelets secret and release the contents, this process may stimulate further platelet recruitment and release. Similar to the critical role of granulocytes in the inflammatory response in sepsis, platelets serve as the toolbox in the inflammation and immune responses in sepsis. Moreover, sepsis-associated thrombocytopenia reflects the severity of inflammatory response [12-14].

The shape and size of platelets could be used as indicators to evaluate the function and activity of platelets [15]. In addition to reflect the proliferation, metabolism of megakaryocyte, the production of platelets also indicates the age of circulating platelets, the ultrastructure and function of platelets. Becchi et al [16] reported that increasing of MPV can be used as an indirect indicator of production and activity disorder of platelets, as well as the indicator for observation of sepsis. The dynamic change of MPV has even greater clinical significance.

CD40L presents inside the inactivated platelets. After activated by thrombin or interacting with CD40, CD40L expresses on the platelet surface, and quickly drops off to be sCD40L in a few minutes or several hours. About 95% of plasma sCD40L mainly derives from the release of activated platelet [17]. Therefore, sCD40L is a plasma marker of platelet activation [18, 19]. Platelets contain abundant intracellular particles, mainly including α-particles, dense bodies and lysosomes. PF4 is a platelet-specific protein in α-particles. When platelets are activated by certain bioactive substances, a large amount of PF4 is released from the platelets, so PF4 is a specific marker of platelet activation *in vivo* [20].

Currently, TPO is considered as an acute phase protein in inflammatory reaction [21]. The expression of TPO varies in different tissues and organs and has different significance in infectious inflammation. The severity of sepsis is closely related with elevated levels of serum TPO. The level of serum TPO can be used as a biological marker of the severity of sepsis. TPO is involved in the pathogenesis of multiple organ dysfunction syndrome [22, 23]. Endogenous TPO is mainly regulated by the total amount of TPO receptors (C-MPL) on the surface of platelets and megakaryocytes. When the platelets or megakaryocytes increase, blood TPO binds to the receptors to be absorbed or degraded. Conversely, when the platelets and megakaryocytes decrease, the TPO level will be elevated [24, 25].

We showed that sPLT decreased and MPV increased significantly in subjects with sepsis-associated thrombocytopenia, compared with healthy controls. Linear correlation analysis showed no significant correlation between MPV and PLT in patients with sepsis-associated thrombocytopenia. In addition, the coagulation indicators, PT, APTT, significantly prolonged and INR increased in patients with sepsis-associated thrombocytopenia, which suggest coagulation disorder in those patients. The plasma levels of sCD40L, PF4 and TPO were higher in patients with sepsis-associated thrombocytopenia. These results suggest that inflammation reactions in patients with sepsis-associated

thrombocytopenia induce the adhesion, aggregation and high activation of platelets, resulting in significant reduction of circulating PLT, the release of growth factors in bone marrow, and the increase of endogenous TPO. The reactive hyperplasia of megakaryocytes in bone marrow produces large and dense platelets, leading to the homogeneous decrease of circulating platelets.

Currently, the approach to treat sepsis-associated thrombocytopenia is limited, except active control of infection and platelet transfusion when necessary. Studies reported that IVIG could improve the severity of sepsis-associated thrombocytopenia, shorten the duration and reduce the severity of inflammation via immune modulation and the defense against infection [26-28]. Thrombopoietin, or rhTPO, is the glycosylated molecule produced by Chinese hamster ovary cells, which contains the full length, endogenous human amino acid sequence. It is consistent with the natural TPO in structure [29]. rhTPO has been approved by CFDA in China. It is indicated in treating solid tumor chemotherapy-induced thrombocytopenia and idiopathic thrombocytopenic purpura. rhTPO increases PLT significantly and reduces the decline of platelet, and shortens the recovery duration of PLT without significant adverse reactions. So far, no neutralizing antibodies against rhTPO have been found in serum [6, 30, 31]. It has been demonstrated [32] that rhTPO combined with conventional therapy can significantly improve the state of severe sepsis-associated thrombocytopenia.

Our research showed that PLT was higher in rhTPO group than IVIG group on d3, d5, d7 and d9. The duration for PLT increasing to $\geq 50\times10^9$/L and $\geq 100\times10^9$/L in rhTPO group was shorter in rhTPO group, which suggests that rhTPO could rapidly improve the thrombocytopenia state of patients with sepsis and shorten the duration of reaching target PLT, and it could reduce the risk of bleeding caused by thrombocytopenia, without affecting patients' coagulation system, hepatic or renal function. The involved mechanism is that rhTPO could widely expand the number of stem cells, accelerate the cell cycle of stem cells, stimulate the survival and proliferation of stem cells [30], expand the pool of committed megakaryocyte progenitors, and promote the formation of megakaryocyte colonies [32]. The drug also possesses the activity of megakaryocyte colony stimulating factor, which induces the proliferation, differentiation and maturation of human megakaryocytes, and production of active platelets.

TPO does not directly activate platelets. However, it can decrease the threshold of platelet activation [33]. This effect may be due to the fact that TPO depends on the activity of phosphatidylinositide 3-kinase, which can phosphorylate the threonine and serine in platelet protein kinase [34]. In the rhTPO tolerance clinical trial, 27 healthy volunteers received multiple doses of rhTPO at 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg. No abnormal platelet aggregation was reported when PLT reached the peak on the 14$^{th}$ day [35]. Our results showed that there is no significant difference in plasma sCD40L, PF4 at each time point between the rhTPO and IVIG group, suggesting that rhTPO has no significant effect on the activation of platelets. Thrombocytopenia is usually accompanied by elevated levels of TPO. In patients with refractory thrombocytopenia, TPO maintains at high level. Despite the constant transcription and translation of TPO, the number of circulating platelets directly determines the circulating levels of TPO [36]. Current results showed that the TPO levels were significantly increased in the 2 groups of patients. However, plasma TPO level was higher in rhTPO group than IVIG group at certain time point. Further study is needed to confirm whether it is related to exogenous TPO.

In summary, sustained and significant reduction of PLT was associated with the high risk of death. The transfusion amount of platelet was less in rhTPO group than IVIG group. Thus, the consumption of medical resources and the risk of related complications due to the transfusion of blood products were reduced. The total hospitalization cost was significantly lower in rhTPO group than IVIG group. No adverse reactions were reported during the observation. These results suggest that rhTPO can effectively improve sepsis-associated thrombocytopenia safely without affecting platelet activation, with no adverse effects on hepatic, renal function or coagulation function. Further study is necessary to clarify the intervention timing, treatment duration and other issues on sepsis-associated thrombocytopenia.

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## Disclosure of conflict of interest

None.

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