Combination of plasma exchange and haemofiltration for treating thrombotic microangiopathy in children

Jun Huang[1], Guangming Chen[1] (ID), Liqiang Ma[2]
and Ting Tan[2]

Abstract

Objective: To investigate the therapeutic effects of a combination of plasma exchange (PE) and haemofiltration (HF) for the treatment for paediatric patients with severe thrombotic microangiopathy (TMA).

Methods: This retrospective study analysed data from paediatric patients who were diagnosed with severe TMA and managed with either supportive therapy and additional PE and HF (PE + HF group) or supportive therapy alone (control group). Demographic and clinical characteristics, haemopurification and blood parameters and survival of the patients were compared between the two groups.

Results: This study enrolled 32 patients: PE + HF group ($n = 15$) and control group ($n = 17$). There were no significant differences between the two groups at baseline, including specific features for differentiating the two categories of TMA. The median time between onset and recovery, defined by the markers of platelet count, lactate dehydrogenase, blood urine nitrogen and hospital stay, were all significantly longer in the control group compared with the PE + HF group. Kaplan–Meier curve analysis demonstrated that the cumulative survival rate was significantly higher in the PE + HF group compared with the control group.

Conclusion: The combination of PE and HF could be considered as one of the treatment choices for TMA.

[1]Department of Paediatrics, 900th Hospital of the Joint Logistics Team of the Chinese PLA, Fuzhou, China
[2]Department of Medicine, 900th Hospital of the Joint Logistics Team of the Chinese PLA, Fuzhou, China

Corresponding author:
Guangming Chen, Department of Paediatrics, 900th Hospital of the Joint Logistics Team of the Chinese PLA, 156 North Road, West Second Ring, Fuzhou, 350025, China.
Email: chenguangming39@126.com

Introduction

Thrombotic microangiopathy (TMA), formerly known as haemolytic uraemic syndrome (HUS), is a life-threatening disease with a triad of microangiopathic haemolytic anaemia, destructive thrombocytopenia and renal failure.[1] The overall incidence of TMA is estimated to be 2.1 per 100 000 people; and it mainly affects children of pre-school age who have an incidence of 6.1 per 100 000.[2,3] The mortality rate is considered to be 2–5%,[4] which usually occurs in the acute phase. Even though 70% of patients recover without permanent sequelae, TMA is the main cause of acute kidney injury in children,[5,6] making the timely treatment for children with suspected TMA essential.

There are two categories of TMA: (i) Shiga toxin-mediated TMA, formerly known as typical HUS; and (ii) complement-mediated TMA, formerly named as atypical HUS.[1] Treatment suggestions are different for these two categories. Shiga toxin-mediated TMA is managed supportively, but the use of antibiotics remains controversial.[7] Plasma exchange (PE) is suggested for the treatment of complement-mediated TMA, but is not considered to be of benefit for Shiga toxin-mediated TMA.[8] However in clinical settings, PE is still used to treat Shiga toxin-mediated TMA,[9,10] and some case reports have described therapeutic success.[11,12]

Plasma exchange is a therapeutic haemopurification procedure in which the blood of the patient is passed through medical apparatus that removes high molecular weight substances and replaces them with a solution such as albumin or fresh frozen plasma, which provides missing essential factors, for example, complement factor H and I in patients with complement-mediated TMA are contained.[1,13] The targeted substances that are planned to be removed include immune complexes, endotoxins, autoantibodies and lipoproteins, all of which are potentially related to immune-mediated disorders.[1,14]

Haemofiltration (HF) is another haemopurification technique that removes excess body fluid across a haemofilter membrane using positive pressure and accumulates substances in patients with severe loss of kidney function that are critically ill.[15] However, HF is rarely reported for the management of both categories of TMA.[7] HF not only helps the removal of toxic substances from the bloodstream, but also improves the balance of electrolytes and acid-base.[16] The therapeutic effects of this normalization of the Na^{2+}, Cl^- and K^+ concentrations by HF have been reported in a canine model of heat stroke; and this is also important in the clinical setting because of the possible reduction of the risk of associated cardiac and neurological complications.[17] In addition, some serum enzymes, for example creatine kinase (CK) and lactate dehydrogenase (LDH), are markedly deceased after HF, indicating the prevention of sequential cellular/organ damage from occurring.[17]

Considering the rationales for both PE and HF, this study investigated the combined use of these two haemopurification methods in critically ill paediatric patients with severe TMA.

Patients and methods

Patients

This retrospective study analysed data from consecutive paediatric patients who were diagnosed with severe TMA and admitted to the paediatric intensive care unit at the 900th Hospital of the Joint Logistics Team of the Chinese PLA, Fuzhou, China between June 2007 and May 2017. Patients were diagnosed with severe TMA according to the following criteria: (i) anaemia (haemoglobin <60 g/l); (ii) low platelet

counts ($< 100\,000/\mu$l); (iii) increased blood urea nitrogen (BUN > 10 mmol/l); (iv) creatinine ($> 150\,\mu$mol/l); (v) haematuria and proteinuria. All of the above should be present when establishing a diagnosis of severe TMA. All patients confirmed no prior history or other concomitant hepatic or renal disorders. Patients were divided into two treatment groups: those who were treated with both supportive therapy and additional PE and HF procedures (PE + HF group), and those that received supportive therapy alone (control group) according to the decisions made by the parents or legal guardians of the children after the detailed treatment plans were explained by the physicians.

The Institutional Review Board of the 900th Hospital of the Joint Logistics Team of the Chinese PLA reviewed and approved this study (no. 200703) and decided that informed consent could be waived due to the observational nature of this study.

Treatment

Supportive therapy included maintaining the balance of fluids and electrolytes, haemodynamic stability and controlling blood pressure. Elective options when necessary included transfusion of red blood cells (RBCs) when haemoglobin <60 g/l and prescription of dipyridamole or diuretics. In the control group, haemodialysis or peritoneal dialysis were performed when patients were anuric for more than 1 day, or there was a rapid increase in BUN, or hyperkalaemia developed, and/or there was oedema, heart failure or resistant hypertension.

For those patients that received haemopurification therapy, PE and HF were started within 24 h of hospital admission and as early as possible. A BM-25 continuous bedside blood purification machine composed of a blood testing module (BM-11) and an ultrafiltration module (BM-14)

(Baxter International, Deerfield, IL, USA) with matching cardiopulmonary bypass tubing, a P_2S plasma separator (membrane areas $= 0.6\,m^2$) and polysulfone hollow fibre haemofilters AV400 (membrane areas $= 0.7\,m^2$) for patients with body weights ≥ 30 kg; or a P_1 dry plasma separator (membrane area $= 0.3\,m^2$) and polysulfone hollow fibre haemofilters AV600 (membrane areas $= 1.4\,m^2$) for patients with body weights <30 kg (all from Fresenius, Bad Homburg, Germany). An 8 Fr double-lumen central venous catheter (Arrow® International, Reading, PA, USA) and 11.5 Fr double lumen haemodialysis catheters (Fresenius) were used. Electrocardiogram and arterial pressures were monitored during PE and HF using a Philips IntelliVue MP40 (Philips Healthcare, Andover, MA, USA).

The procedures were modified based on previous studies.[14,17] PE was conducted first followed by HF in an intermittent haemofiltration manner. Every complete procedure was undertaken over an interval of 1–3 days depending on the results of individual patient's blood and biochemical tests. The catheter was placed via the femoral vein or subclavian vein for paediatric patients due to the higher risk of them falling out and the subsequent bleeding if placed via the internal jugular vein. The tubing was initially flushed with 25 U/ml heparin sodium saline for 30 min. A pre-prepared 100–150 ml suspension of RBCs was then infused. Heparin was administered intra-arterially 1 min before haemofiltration with 0.20–0.40 mg/kg, the speed was maintained at 0.10–0.20 mg/kg per h, adjusted according to the transmembrane and venous pressure as well as the coagulation function. PE was conducted using fresh frozen plasma as the replacement fluid by the post-replacement method. The blood flow rate was 45–140 ml/min, the plasma exchange rate was 400–1600 ml/h, and the plasma separation rate, which was

calculated as plasma exchange rate/(blood flow rate $\times$ 60) $\times$ 100, was 12.80–25.00%. The volume of exchange was based on the guidelines and set as 1 to 2 plasma volume exchanges per patient;[1] and calculated according to the following formula: plasma volume = (1 – hematocrit) $\times$ (b + c $\times$ W), where W = body weight (kg), b = constant (1530 for males and 864 for females), and c = constant (41 for males and 47.2 for females). The volume of exchange was 880–2687 ml in this study. A 10% calcium gluconate (1 ml/100 ml plasma exchanged) solution was administered to prevent hypocalcaemia. HF was conducted using the pre-replacement method: the exchange rate was 900–4000 ml/h; and the volume of exchange was 11.00–56.80 l for 9–22 h. The net fluid removed (the volume of substitution fluids was not included) was 0–1200 ml. During HF, a suspension of RBCs was infused when necessary. Heparin was ceased 15 min before the end of HF.

Outcome measurements

The demographic characteristics were recorded for all patients. Arterial and venous blood samples were collected at days 1, 3, 5 and 10 after admission. Heparin-anticoagulated arterial blood samples were used for the assessment of blood gas parameters immediately using an ABL800 FLEX blood gas analyser (Radiometer, Thalwil, Switzerland). Ethylenediamine tetra-acetic acid anticoagulated venous blood samples were used to assess the complete blood count within 2 h using a Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan). Sodium citrate-anticoagulated venous blood samples were used for coagulation testing using a CS-5100 Haemostasis System analyser (Sysmex). Serum was collected from venous blood samples within 2 h and biochemistry analyses were conducted within

4 h using a Cobas c 702 modular analyser (Roche Diagnostics, Mannheim, Germany). Before assessment, serum was stored at 4 °C. The biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, BUN, cholinesterase, CK, creatinine, α-hydroxybutyrate dehydrogenase (α-HBDH), LDH and the concentrations of electrolytes including Na^+, K^+, Cl^- and Ca^{2+}. The Paediatric Clinical Illness Score (PCIS) was assessed at days 1, 3, 5 and 10 after admission.[18] A PCIS > 90 was defined as 'not critically ill', 90–70 as 'critically ill' and <70 as 'very critically ill'.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows®, Version 24.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as the median and interquartile range (P_{25}–P_{75}) and categorical variables are presented as number of patients (%). The comparison between the PE + HF group and the control group was performed using Mann–Whitney U-test for continuous variables and using χ^2-test or Fisher's exact test for categorical variables. The comparisons among hospital admission (baseline), 3, 5 and 10 days after treatment were performed using Friedman test for continuous variables and Cochran's Q test for categorical variables. The results were Bonferroni corrected to take into account the number of pairwise comparisons. Kaplan–Meier curve analysis with log-rank test was performed to compare the cumulative survival rate between the PE + HF and control groups. Propensity score was constructed by the demographic and vital sign variables, including age, sex, weight, PCIS, heart rate, respiratory rate, systolic blood pressure and diastolic blood pressure, and used to adjust the survival effect for the group comparison.

A *P*-value <0.05 was considered statistically significant.

Results

This retrospective study enrolled 32 patients: 15 patients in the PE + HF group and 17 patients in the control group. There were no significant differences between the two groups at baseline, including specific features for differentiating the two categories of TMA (Table 1). The median time between onset and recovery, defined as platelet count > 150×10^9/l, LDH <500 U/l and BUN <8.2 mmol/l, and duration of hospital stay were all significantly longer in the control group compared with the PE + HF group ($P \leq 0.033$ for all comparisons).

The volume of RBC suspension significantly decreased in the fourth round of haemopurification compared with the first round of haemopurification (median, 150 ml versus 350 ml; $P = 0.027$) (Table 2). The volume of HF exchange significantly decreased in the third round of haemopurification compared with the first round of haemopurification (median, 21 ml versus 25 ml; $P = 0.048$). The blood flow rate, venous pressures, transmembrane pressures, durations of the procedures, exchange rates and plasma separation rate of PE and volume of net fluid removed for HF were all consistent among the different rounds of treatment.

The severity of TMA was measured using PCIS, cholinesterase, LDH and α-HBDH levels (Table 3). At the end of treatment (day 10), both the control and PE + HF groups showed significant improvements in all of these markers compared with baseline ($P < 0.05$ for all comparisons). For PCIS, LDH and α-HBDH levels, the PE + HF group demonstrated significantly earlier reductions in severity, represented by significant differences between the PE + HF and control groups

at days 3, 5 and 10 ($P < 0.05$ for all comparisons). Compared with baseline, PCIS, LDH and α-HBDH levels were all significantly improved in the PE + HF group from day 5 to day 10; in the control group, the significance from baseline was delayed to day 10. For cholinesterase, there were no significant differences between the PE + HF and control groups at each time-point after treatment. Cholinesterase level was significantly increased at days 5 and 10 compared with baseline in the PE + HF group ($P \leq 0.005$ for both comparisons). In the control group, cholinesterase level was significantly increased at day 10 after treatment compared with baseline ($P = 0.045$).

At the end of treatment (day 10), all of the parameters, except creatinine, were significantly improved in both the PE + HF and control groups compared with baseline ($P < 0.05$ for all comparisons) (Table 4). The creatinine level demonstrated no significant differences at all of the time-points compared with baseline in the control group. RBC counts, haemoglobin, CK-MB, total and direct bilirubin were significantly better at the earliest time-point (day 3 after treatment) in the PE + HF group compared with the control group ($P \leq 0.001$ for all comparisons). The significant differences between the two treatment groups were delayed to day 5 for platelet count, BUN, CK and ALT ($P < 0.05$ for all comparisons). Neutrophil level, creatinine and AST only showed significant differences between the two groups at day 10 ($P < 0.05$ for all comparisons).

Comparing the differences between the two groups at each time-point after treatment, the abnormal rates of Na$^+$ and Cl$^-$ significantly decreased in the PE + HF group compared with the control group at 10 days ($P = 0.038$ for both comparisons); and the abnormal rate of Cl$^-$ significantly decreased in the PE + HF group compared with the control group 5 days after treatment ($P = 0.024$) (Table 5). Comparing the

Table 1. Baseline demographic, clinical characteristics and outcomes between two groups of paediatric patients with severe thrombotic microangiopathy (TMA) treated with either supportive therapy alone (control) or with a combination of plasma exchange (PE) and haemofiltration (HF).

Characteristics/outcomes	PE + HF group $n = 15$	Control group $n = 17$	Statistical significance[a]
Demographic			
Age, years	3.83 (2.58–6.08)	4.67 (1.75–7.16)	NS
Sex			NS
Male	10 (66.67%)	11 (64.71%)	
Female	5 (33.33%)	6 (35.29%)	
Weight, kg	15.6 (13.0–20.0)	16.5 (13.5–23.5)	NS
Vital signs and haematological			
PCIS	72 (66–76)	76 (62–76)	NS
Heart rate, beats/min	108 (88–120)	100 (92–115)	NS
Respiratory rate, breaths/min	26 (21–35)	25 (22–32)	NS
Systolic blood pressure, mmHg	110 (95–125)	115 (98–125)	NS
Diastolic blood pressure, mmHg	65 (56–85)	73 (61–86)	NS
Mean arterial pressure , mmHg	79 (69–96)	87 (73–100)	NS
RBC, $\times 10^{12}$/l	1.86 (1.62–1.88)	1.87 (1.71–1.96)	NS
Haemoglobin, g/l	53 (34–58)	52 (48–55)	NS
PLT, $\times 10^{9}$/l	36 (21–51)	38 (32–50)	NS
WBC, $\times 10^{9}$/l	18.42 (14.12–22.12)	15.04 (10.70–21.50)	NS
Neutrophil, %	58.00 (54.20–69.90)	70.30 (61.24–78.20)	NS
TMA-related			
Decreased C3	5 (62.50)[b]	7 (70.00)[c]	NS
With respiratory infection	5 (33.33)	6 (35.29)	NS
With gastroenteritis-like symptoms	6 (40.00)	7 (41.18)	NS
Biochemical			
Cholinesterase, U/l	3843 (1215–4879)	3583 (2143–4875)	NS
LDH, U/l	2567 (1458–3281)	2445 (1845–2978)	NS
α-HBDH, U/l	1542 (1148–2120)	1758 (1568–2165)	NS
BUN, mmol/l	35.2 (21.3–43.2)	34.2 (23.6–45.2)	NS
Creatinine, μmol/l	370 (285–385)	368 (285–412)	NS
CK, U/l	321 (256–395)	276 (189–389)	NS
CK-MB, U/l	65 (58–87)	96 (69–125)	NS
Total bilirubin, μmol/l	37.6 (28.7–56.6)	35.5 (25.9–49.2)	NS
Direct bilirubin, μmol/l	12.0 (10.2–21.5)	12.5 (8.3–16.3)	NS
ALT, U/l	65 (48–84)	65 (56–75)	NS
AST (U/L)	134 (98–191)	142 (110–159)	NS
Electrolytes and blood gases			
K^{+}, mmol/l	4.6 (3.4–5.1)	4.6 (3.8–5.0)	NS
Na^{+}, mmol/l	129 (126–135)	130 (127–134)	NS
Cl^{-}, mmol/l	86 (81–95)	94 (86–98)	NS
Ca^{2+}, mmol/l	2.10 (1.91–2.20)	2.09 (1.87–2.20)	NS
pH	7.32 (7.24–7.37)	7.32 (7.24–7.38)	NS
$PaCO_2$, mmHg	25.6 (23.6–31.2)	25.3 (22.5–32.8)	NS
PaO_2, mmHg	78 (75–87)	78 (69–89)	NS
HCO_3^{-}, mmol/l	18.6 (15.3–20.7)	17.8 (14.3–21.4)	NS
Base excess, mmol/l	−10 (−13– −6)	−10 (−12– −2)	NS

(continued)

Table 1. Continued.

Characteristics/outcomes	PE + HF group n =15	Control group n =17	Statistical significance[a]
Outcomes			
Time between onset and hospital admission, hr	112 (85–128)	98 (85–125)	NS
Time between admission and first haemopurification, h	12 (9–14)	–	–
Time between onset and first haemopurification, h	124 (96–144)	–	–
Time between onset and platelet count $>150 \times 10^9$/l, days	13.5 (12.0–16.0)	18.5 (14.5–23.0)	$P = 0.015$
Time between onset and LDH < 500 U/l, days	19 (17–21)	28 (24–36)	$P = 0.002$
Time between onset and BUN < 8.2 mmol/l, days	20.5 (19.0–26.0)	35.5 (25.5–43.5)	$P = 0.001$
Hospital stay, days	19 (15–27)	28 (22–38)	$P = 0.033$

Data presented as median (interquartile range) or n of patients (%).
[a]The PE + HF and control group comparisons were performed using Mann–Whitney U-test for continuous variables and χ^2-test or Fisher's exact test for categorical variables.
[b]Only eight patients had available data; [c]Only 10 patients had available data.
PCIS, Paediatric Clinical Illness Score; RBC, red blood cell; PLT, platelet; WBC, white blood cell; C3, complement component 3; LDH, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; BUN, blood urea nitrogen; CK, creatine phosphokinase; CK-MB, creatine kinase MB isoenzyme; ALT, alanine transaminase; AST, aspartate transaminase; NS, no significant between-group difference ($P \geq 0.05$).

differences among different time-points in the PE + HF group, the abnormal rates of Na^+ and Cl^- significantly decreased after 5 and 10 days of treatment compared with baseline ($P \leq 0.013$ for all comparisons). No significant differences in the abnormal rates of Na^+ and Cl^- were found between the different time-points in the control group.

Comparing the differences between the two groups at each time-point after treatment, the abnormal rates of pH, HCO_3^- and base excess decreased more significantly in the PE + HF group than the control group 3 days after treatment ($P \leq 0.016$ for all comparisons) (Table 5). Comparing the differences among the different time-points in the PE + HF group, the abnormal rates of pH, $PaCO_2$, HCO_3^- and base excess significantly decreased after treatment at day 3 and 10 compared with baseline ($P \leq 0.020$ for all comparisons); the abnormal rate of pH and HCO_3^- significantly decreased 5 days after treatment compared with baseline ($P \leq 0.012$ for both comparisons). Comparing the differences among different time-points in the control group, the abnormal rate of pH, $PaCO_2$ and HCO_3^- significantly decreased 10 days after treatment compared with baseline ($P \leq 0.031$ for all comparisons); and the abnormal rate of pH and HCO_3^- significantly decreased 10 days after treatment compared with day 3 ($P \leq 0.026$ for both comparisons).

There were 14 of 15 (93.3%) patients and nine of 17 (52.9%) patients that survived at the last follow-up in the PE + HF and the control groups, respectively. Kaplan–Meier curve analysis demonstrated that the cumulative survival rate was significantly higher in the PE + HF group compared with the

Table 2. Haemopurification parameters during different rounds of the plasma exchange (PE) and haemofiltration (HF) procedures in paediatric patients with severe thrombotic microangiopathy.

	Treatment rounds				Statistical significance[a]
	First n = 15	Second n = 14)	Third n = 12	Fourth n = 3	
Suspension of red blood cells, ml	350 (200–450)	100 (100–150)	150 (150–200)	150 (100–200)*	P = 0.039
Blood flow rate, ml/min	70 (60–80)	70 (70–80)	70 (68–75)	70 (50–80)	NS
Venous pressures during PE, mmHg	30 (25–40)	35 (20–50)	40 (30–43)	40 (20–40)	NS
Venous pressures during HF, mmHg[†]	50 (40–70)	60 (50–60)	60 (50–60)	–	NS
Transmembrane pressures during PE, mmHg[†]	50 (30–60)	50 (40–60)	60 (50–65)	50 (10–60)	NS
Transmembrane pressures during HF, mmHg[†]	90 (50–100)	85 (70–90)	85 (70–110)	–	NS
Duration of PE, h	2.2 (2.1–2.3)	2.2 (2.0–2.3)	2.0 (2.0–2.4)	2.0 (1.9–2.2)	NS
Volume of PE exchange, l	1689 (1120–2120)	1804 (1457–2040)	1648 (1160–1888)	2010 (930–2015)	NS
PE exchange rate, ml/h	800 (600–1100)	900 (700–1000)	800 (650–950)	1000 (500–1000)	NS
PE plasma separation rate, %	20.00 (16.67–21.43)	18.90 (15.39–21.43)	17.20 (15.15–20.26)	20.80 (16.67–23.81)	NS
Duration of HF, h[†]	15 (11–16)	13 (12–15)	12 (10–14)	–	NS
Volume of HF exchange, l[†]	25 (20–35)	26 (23–29)	21 (18–29)*	–	NS
HF exchange rate, ml/h[†]	2000 (1500–2000)	1800 (1600–2000)	2000 (1500–2000)	–	NS
Net fluid removed for HF, ml[†]	400 (300–600)	250 (100–400)	100 (0–200)	–	NS

Data presented as median (interquartile range).

[a]Friedman test.

*$P < 0.05$ indicates a significant difference compared with the first round of treatment; NS, no significant difference ($P \geq 0.05$).

[†]Represents a significant difference across the first, second and third rounds of treatment.

Table 3. Severity of thrombotic microangiopathy before and after treatment in two groups of paediatric patients treated with either supportive therapy alone (control) or with a combination of plasma exchange (PE) and haemofiltration (HF).

| | Baseline | Time after treatment | | | Statistical significance[a] |
		3 days	5 days	10 days	
PCIS					
PE + HF	72 (66–76)	78 (72–82)	80 (74–84)*	89 (86–92)*†‡	$P < 0.001$
Control	76 (62–76)	72 (64–74)#	76 (66–78)#	83 (75–84)#*†	$P < 0.001$
Cholinesterase, U/l					
PE + HF	3843 (1215–4879)	4784 (3645–6069)	6250 (5678–6978)*	6112 (5412–7896)*	$P = 0.001$
Control	3583 (2143–4875)	4578 (3245–5148)	5048 (4520–6215)	5445 (3891–6483)*	$P = 0.045$
LDH, U/l					
PE + HF	2567 (1458–3281)	1326 (897–2141)	1015 (784–1254)*	266 (215–462)*†	$P < 0.001$
Control	2445 (1845–2978)	1974 (1545–2512)#	1358 (1012–2167)#	683 (514–928)#*†‡	$P < 0.001$
α-HBDH, U/l					
PE + HF	1542 (1148–2120)	845 (742–1031)	827 (621–956)*	105 (59–201)*†	$P < 0.001$
Control	1758 (1568–2165)	1578 (1245–2014)#	1047 (845–1687)#	496 (323–646)#*†‡	$P < 0.001$

Data presented as median (interquartile range).
[a]Friedman test.
*$P < 0.05$ indicates a significant difference compared with baseline.
†$P < 0.05$ indicates a significant difference compared with day 3.
‡$P < 0.05$ indicates a significant difference compared with day 5.
#$P < 0.05$ indicates a significant difference between the PE + HF group and control group.
PCIS, Paediatric Clinical Illness Score; LDH, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase.

control group ($P = 0.015$) (Figure 1A). A propensity score constructed to adjust the survival effect demonstrated that the adjusted cumulative survival rate of the PE + HF group was still higher compared with the control group, but the difference was not statistically significant (Figure 1B).

Discussion

This current retrospective study intensively monitored many parameters due to the critical condition of the paediatric patients that were enrolled in the study. In addition, these current results support the rationale for the therapeutic effects of this haemopurification procedure. Compared with transfusion, using an infusion of a suspension of RBCs allowed for the faster administration of a larger volume to treat the anaemia in paediatric patients with TMA; with the blood volume and haemodynamics being controlled well by HF. Using HF alone can improve and maintain fluid balance, electrolyte status and acid-base balance, which reduces impairments to the circulatory system, liver and heart; and is probably also related to a reduction in the risk of more severe complications like multiple organ failure.[12,19] Keeping a higher central venous pressure (8–10 cmH$_2$O; normal range for paediatric patients: 5.79–11.08 cmH$_2$O) during HF could contribute to the recovery of renal function due to the avoidance of hypoperfusion and ischaemia.

The major limitation of this current study was that it was not able to specify the two TMA categories of the paediatric patients because the essential clinical examinations for differential diagnosis were not available to the physicians. As the diagnostic categories of patients represent different pathologies, the effects of PE and HF might be different depending upon the

Table 4. Haematological and biochemical parameters before and after treatment in two groups of paediatric patients treated with either supportive therapy alone (control) or with a combination of plasma exchange (PE) and haemofiltration (HF).

| | Baseline | Time after treatment | | | Statistical significance[a] |
		3 days	5 days	10 days	
RBC, $\times 10^{12}$/l					
PE + HF	1.86 (1.62–1.88)	2.66 (2.45–3.35)	2.94 (2.68–3.45)*	3.50 (3.12–3.76)*†	$P < 0.001$
Control	1.87 (1.71–1.96)	2.25 (1.96–2.45)#	2.53 (2.19–2.57)#*	2.76 (2.38–3.09)#*†	$P < 0.001$
Haemoglobin, g/l					
PE + HF	53 (34–58)	76 (61–98)*	81 (64–98)*	105 (89–115)*†	$P < 0.001$
Control	52 (48–55)	58 (56–60)#	60 (55–72)#*	71 (64–91)#*†	$P < 0.001$
PLT, $\times 10^9$/l					
PE + HF	36 (21–51)	47 (35–57)	81 (68–121)*†	154 (125–195)*†	$P < 0.001$
Control	38 (32–50)	45 (30–56)	59 (45–78)#*	129 (69–172)#*†	$P < 0.001$
WBC, $\times 10^9$/l					
PE + HF	18.42 (14.12–22.12)	14.23 (10.36–18.14)	10.55 (7.85–12.50)	7.57 (5.80–8.32)*†	$P = 0.001$
Control	15.04 (10.70–21.50)	14.21 (8.72–17.91)	13.28 (9.46–18.23)	8.32 (6.65–13.34)*	$P = 0.016$
Neutrophil, %					
PE + HF	58 (540–70)	57 (51–63)	56 (54–62)	41 (35–47)*†	$P < 0.001$
Control	70.3 (61.2–78.2)	65.0 (57.6–72.6)	61.5 (56.0–75.0)	57.7 (47.6–65.4)#*	$P = 0.022$
BUN, mmol/l					
PE + HF	35.2 (21.3–43.2)	28.1 (20.3–35.2)	21.0 (13.2–24.3)*	10.9 (9.5–14.6)*†	$P < 0.001$
Control	34.2 (23.6–45.2)	31.7 (23.5–35.4)	25.3 (23.4–32.9)#	19.2 (16.8–23.3)#*†	$P = 0.001$
Creatinine, μmol/l					
PE + HF	370 (285–385)	297 (268–345)	241 (178–268)	139 (88–154)*†	$P < 0.001$
Control	368 (285–412)	325 (254–377)	278 (245–352)	266 (174–300)#	NS
CK, U/l					
PE + HF	321 (256–395)	225 (154–326)	143 (121–175)*	69 (58–95)*†	$P < 0.001$
Control	276 (189–389)	286 (212–358)	231 (156–285)#	104 (87–152)#*†‡	$P < 0.001$
CK-MB, U/l					
PE + HF	65 (58–87)	59 (38–78)	46 (36–55)*	30 (21–38)*†	$P < 0.001$
Control	96 (69–125)	87 (75–108)#	78 (65–98)#	47 (34–62)#*†	$P = 0.012$
Total bilirubin, μmol/l					
PE + HF	37.6 (28.7–56.6)	19.8 (12.5–23.6)*	13.1 (11.5–16.3)*	13.4 (12.3–17.5)*	$P < 0.001$
Control	35.5 (25.9–49.2)	25.3 (21.5–35.2)#	19.3 (15.3–24.3)#*†	12.3 (9.5–15.3)*†	$P < 0.001$
Direct bilirubin, μmol/l					
PE + HF	12.0 (10.2–21.5)	5.6 (4.0–11.2)*	5.0 (4.0–6.2)*	5.0 (5.0–5.2)*	$P < 0.001$
Control	12.5 (8.3–16.3)	9.5 (8.0–12.6)#	6.0 (5.0–8.0)*	5.3 (4.0–6.3)*†	$P < 0.001$
ALT, U/l					
PE + HF	65 (48–84)	51 (37–69)	40 (25–45)*	29 (15–36)*†	$P < 0.001$
Control	65 (56–75)	56 (45–85)	45 (37–69)#	40 (35–50)#*	$P = 0.028$
AST, U/l					
PE + HF	134 (98–191)	102 (68–145)	67 (48–89)*	59 (36–65)*†	$P < 0.001$
Control	142 (110–159)	105 (78–156)	65 (58–142)	77 (68–88)#*†	$P = 0.003$

Data presented as median (interquartile range).

[a]Friedman test; NS, no significant difference ($P \geq 0.05$).

*$P < 0.05$ indicates a significant difference compared with baseline.

†$P < 0.05$ indicates a significant difference compared with day 3.

#$P < 0.05$ indicates a significant difference between the PE + HF group and control group.

RBC, red blood cell; PLT, platelet; WBC, white blood cell; BUN, blood urea nitrogen; CK, creatine phosphokinase; CK-MB, creatine kinase MB isoenzyme; ALT, alanine transaminase; AST, aspartate transaminase.

Table 5. The abnormal distribution of electrolytes and blood gases before and after treatment in two groups of paediatric patients treated with either supportive therapy alone (control; $n = 17$) or with a combination of plasma exchange (PE) and haemofiltration (HF) ($n = 15$).

| | Baseline | Time after treatment | | | Statistical significance[b] |
		3 days	5 days[a]	10 days[a]	
K^+					
PE + HF	7 (46.67%)	2 (13.33%)	1 (7.14%)	1 (7.14%)	$P = 0.037$
Control	7 (41.18%)	6 (35.29%)	5 (31.25%)	6 (37.50%)	NS
Na^+					
PE + HF	12 (80.00%)	6 (40.00%)	3 (21.43%)*	2 (14.29%)*	$P = 0.002$
Control	14 (82.35%)	12 (70.59%)	9 (56.25%)	8 (50.00%)[#]	$P = 0.072$
Cl^-					
PE + HF	12 (80.00%)	7 (46.67%)	3 (21.43%)*	2 (14.29%)*	$P = 0.002$
Control	13 (76.47%)	9 (52.94%)	10 (62.50%)[#]	8 (50.00%)[#]	NS
Ca^{2+}					
PE + HF	6 (40.00%)	3 (20.00%)	1 (7.14%)	2 (14.29%)	NS
Control	7 (41.18%)	6 (35.29%)	6 (37.50%)	5 (31.25%)	NS
pH					
PE + HF	11 (73.33%)	4 (26.67%)*	2 (14.29%)*	1 (7.14%)*	$P < 0.001$
Control	14 (82.35%)	13(76.47%)[#]	8 (50.00%)	4 (25.00%)*[†]	$P = 0.003$
$PaCO_2$					
PE + HF	13 (86.67%)	6 (40.00%)*	6 (42.86%)	3 (21.43%)*	$P = 0.001$
Control	13 (76.47%)	12 (70.59%)	10 (62.50%)	5 (31.25%)*	$P = 0.037$
PaO_2					
PE + HF	9 (60.00%)	4 (26.67%)	2 (14.29%)	3 (21.43%)	NS
Control	9 (52.94%)	8 (47.06%)	6 (37.50%)	3 (18.75%)	NS
HCO_3-					
PE + HF	12 (80.00%)	3 (20.00%)*	3 (21.43%)*	2 (14.29%)*	$P = 0.001$
Control	13 (76.47%)	11 (64.71%)[#]	7 (43.75%)	2 (12.50%)*[†]	$P = 0.002$
Base excess					
PE + HF	12 (80.00%)	4 (26.67%)*	5 (35.71%)	3 (21.43%)*	$P = 0.005$
Control	13 (76.47%)	13 (76.47%)[#]	9 (56.25%)	7 (43.75%)	NS

Data presented as n of patients (%) with abnormal values.

[a]Missing data for one patient for days 5 and 10 in both groups: PE + HF ($n = 14$) and control group ($n = 16$).

[b]Cochran's Q test; NS, no significant difference ($P \geq 0.05$).

*$P < 0.05$ indicates a significant difference compared with baseline.

[†]$P < 0.05$ indicates a significant difference compared with day 3.

[#]$P < 0.05$ indicates a significant difference between the PE + HF group and control group.

diagnostic category and warrants further investigation. On a practical level, it is a time-consuming process to confirm the differential diagnosis of the two categories of TMA and many hospitals worldwide face the same difficulty. The clinical presentation was similar between the two study groups in this current study, including the rates of respiratory infection and gastroenteritis-like symptoms, which might be of use for specifying the TMA category. This suggests that the distribution of the two TMA categories was similar in the two study groups. Thus, this experimental strategy of supportive treatment with additional haemopurification may offer a more
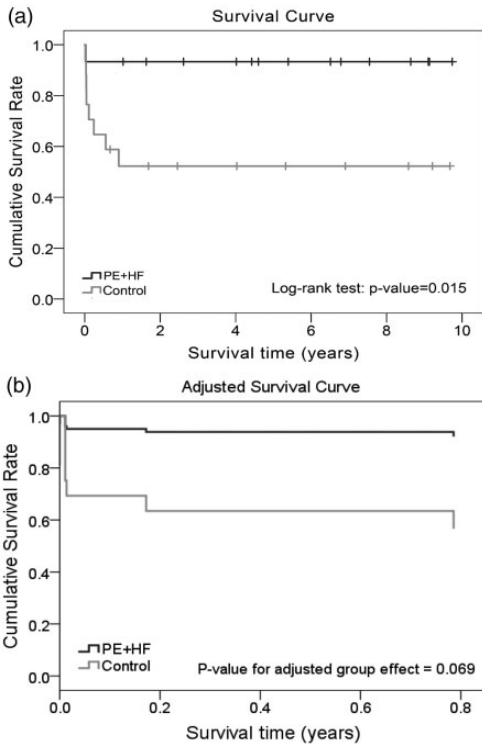
Figure 1. Kaplan–Meier survival curves for two groups of paediatric patients with severe thrombotic microangiopathy treated with either supportive therapy alone (control) or with a combination of plasma exchange (PE) and haemofiltration (HF). (a) original; (b) adjusted by propensity score.

practical and easier approach that would enable rapid medical decision-making in emergency departments or intensive care units for critically ill patients. PE is considered ineffective for the treatment of Shiga toxin-mediated TMA.[4,20] However, the remission and survival of the PE + HF group was significantly better than that of the control group in this current study. It should be noted that after adjusting for the propensity score constructed using the demographic and vital sign variables of the patients, the survival rate was still higher in the PE + HF group, but it did not reach statistical significance. This may

reflect the small sample size, but the effects of the heterogeneity of the patients in each group cannot be ruled out. The retrospective design also limited data acquisition. A better study design with clearly specified TMA categories is required in the future.

The severity of TMA was monitored using PCIS, cholinesterase, LDH and α-HBDH. PCIS is a widely used scoring system in China, which was developed by the Chinese Paediatric Society and the Chinese Emergency Medicine Society.[18] The items include heart rate, blood pressure, blood gases, biochemistry and electrolytes, which aims to evaluate the physical condition of paediatric patients. The PCIS has not been translated into English, or validated in other countries, and a tool that transfers data from the PCIS to more internationally familiar scores, such as the Paediatric Index of Mortality 2, remains unavailable. This limitation might result in international researchers in this field failing to understand the severity of TMA that the PCIS represents. Due to the retrospective design of this current study, the PCIS data can only be presented with a translated supplementary table as a reference. Cholinesterase has been reported as a prognostic or severity marker for several diseases, including severe injury,[21] sepsis,[22] dementia,[23] and acute organophosphate pesticide poisoning.[24] LDH and α-HBDH levels are associated with renal damage and infection.[25,26] Although these biomarkers are non-specific and increase in many other diseases, they can be easily measured using automated haematological analysers, which are routinely available in most healthcare settings. From these current results, all four parameters have the potential to be developed as markers or individual items of a combined scoring system to simply evaluate the severity of TMA when monitoring the therapeutic effects of emergency treatment.

Eculizumab is a humanized monoclonal antibody that inhibits C5 activation and blocks the production of the proinflammatory C5a anaphylatoxin, which is recommended for the treatment of complement-mediated TMA both in paediatric and adult patients.[27] However, financial constraints and non-availability of eculizumab limit its use in many clinical settings. The current findings, along with those of a previously published study that investigated 30 paediatric patients with complement-mediated TMA that were treated with PE,[28] suggest that haemopurification might be a viable and more cost-effective alternative to eculizumab.

This current study had several other limitations that should be acknowledged in addition to those described above. These include the relatively small sample size and the retrospective study design. For paediatric patients, smaller plasma separators and haemofilters should be used for decreasing the volume of the extracorporeal circulation. However due to limited resources, pre-prepared 100–150 ml suspensions of RBCs were infused instead. As discussed above, the outcomes should be analysed in groups stratified according to the category of TMA, with the aim of understanding the mechanisms involved more clearly so that appropriate modifications can be made to the procedures as required.

In conclusion, the treatment of paediatric patients with severe TMA using a combination of PE and HF significantly increased their survival compared with routine supportive treatment. These current findings suggest that PE + HF could be considered as one of the treatment choices for patients with TMA.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

ORCID iD

Guangming Chen   https://orcid.org/0000-0001-8295-8754

References

1. Schwartz J, Padmanabhan A, Aqui N, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the writing committee of the American Society for Apheresis: the seventh special issue. *J Clin Apher* 2016; 31: 149–162. DOI: 10.1002/jca.21470.
2. Ruggenenti P, Noris M and Remuzzi G. Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. *Kidney Int* 2001; 60: 831–846. DOI: 10.1046/j.1523-1755.2001.060003831.x.
3. Tarr PI, Gordon CA and Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *Lancet* 2005; 365: 1073–1086. DOI: 10.1016/S0140-6736(05)71144-2.
4. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* 2003; 290: 1360–1370. DOI: 10.1001/jama.290.10.1360.
5. Williams DM, Sreedhar SS, Mickell JJ, et al. Acute kidney failure: a pediatric experience over 20 years. *Arch Pediatr Adolesc Med* 2002; 156: 893–900.
6. Jenssen GR, Vold L, Hovland E, et al. Clinical features, therapeutic interventions and long-term aspects of hemolytic-uremic syndrome in Norwegian children: a nationwide retrospective study from 1999-2008. *BMC Infect Dis* 2016; 16: 285. DOI: 10.1186/s12879-016-1627-7.
7. Wurzner R, Riedl M, Rosales A, et al. Treatment of enterohemorrhagic Escherichia coli-induced hemolytic uremic syndrome (eHUS). *Semin Thromb Hemost*

2014; 40: 508–516. DOI: 10.1055/s-0034-1375298.

8. Kennedy J, Simmonds L, Orme R, et al. An unusual case of Escherichia coli O157:H7 infection with pseudomembranous colitis-like lesions associated with haemolytic-uraemic syndrome and neurological sequelae. *BMJ Case Rep* 2017; 2017: pii: bcr-2016-218586. DOI: 10.1136/bcr-2016-218586.

9. Paglialonga F, Schmitt CP, Shroff R, et al. Indications, technique, and outcome of therapeutic apheresis in European pediatric nephrology units. *Pediatr Nephrol* 2015; 30: 103–111. DOI: 10.1007/s00467-014-2907-3.

10. Sengul Samanci N, Ayer M, Gursu M, et al. Patients treated with therapeutic plasma exchange: a single center experience. *Transfus Apher Sci* 2014; 51: 83–89. DOI: 10.1016/j.transci.2014.10.006.

11. Perez-Cruz FG, Villa-Diaz P, Pintado-Delgado MC, et al. Hemolytic uremic syndrome in adults: a case report. *World J Crit Care Med* 2017; 6: 135–139. DOI: 10.5492/wjccm.v6.i2.135.

12. Mijatovic D, Blagaic A and Zupan Z. Case report: severe form of hemolytic-uremic syndrome with multiple organ failure in a child: a case report. *F1000Res* 2014; 3: 79. DOI: 10.12688/f1000research.2546.2.

13. Nakanishi T, Suzuki N, Kuragano T, et al. Current topics in therapeutic plasmapheresis. *Clin Exp Nephrol* 2014; 18: 41–49. DOI: 10.1007/s10157-013-0838-0.

14. Wang CF, Nie XJ, Chen GM, et al. Early plasma exchange for treating ricin toxicity in children after castor bean ingestion. *J Clin Apher* 2015; 30: 141–146. DOI: 10.1002/jca.21351.

15. Saito A. Current progress in blood purification methods used in critical care medicine. *Contrib Nephrol* 2010; 166: 100–111. DOI: 000314858 [pii] 10.1159/000314858.

16. Wakino S, Hori S, Mimura T, et al. Heat stroke with multiple organ failure treated with cold hemodialysis and cold continuous hemodiafiltration: a case report. *Ther Apher Dial* 2005; 9: 423–428. DOI: TAP321 [pii] 10.1111/j.1744-9987.2005.00321.x.

17. Chen GM, Xu HN, Gao LF, et al. Effects of continuous haemofiltration on serum enzyme concentrations, endotoxemia, homeostasis and survival in dogs with severe heat stroke. *Resuscitation* 2012; 83: 657–662. DOI: 10.1016/j.resuscitation.2011.11.003.

18. Fan XM. Clinical application of simplified pediatric critical illness scoring system. *Zhonghua Er Ke Za Zhi* 2003; 41: 565–569 [Article in Chinese, English abstract].

19. Andreoli SP. Management of acute kidney injury in children: a guide for pediatricians. *Paediatr Drugs* 2008; 10: 379–390. DOI: 10.2165/0148581-200810060-00005.

20. Bambauer R, Latza R and Schiel R. Therapeutic apheresis in the treatment of hemolytic uremic syndrome in view of pathophysiological aspects. *Ther Apher Dial* 2011; 15: 10–19. DOI: 10.1111/j.1744-9987.2010.00903.x.

21. Zhang QH, Li AM, He SL, et al. Serum total cholinesterase activity on admission is associated with disease severity and outcome in patients with traumatic brain injury. *PLoS One* 2015; 10: e0129082. DOI: 10.1371/journal.pone.0129082.

22. Zivkovic AR, Decker SO, Zirnstein AC, et al. A sustained reduction in serum cholinesterase enzyme activity predicts patient outcome following sepsis. *Mediators Inflamm* 2018; 2018: 1942193. DOI: 10.1155/2018/1942193.

23. Zou J, Chen Z, Liang C, et al. Trefoil factor 3, cholinesterase and homocysteine: potential predictors for Parkinson's disease dementia and vascular parkinsonism dementia in advanced stage. *Aging Dis* 2018; 9: 51–65. DOI: 10.14336/ad.2017.0416.

24. Yuan S, Gao Y, Ji W, et al. The evaluation of acute physiology and chronic health evaluation II score, poisoning severity score, sequential organ failure assessment score combine with lactate to assess the prognosis of the patients with acute organophosphate pesticide poisoning. *Medicine (Baltimore)* 2018; 97: e10862. DOI: 10.1097/md.0000000000010862.

25. Sun J, Su J, Xie Y, et al. Plasma IL-6/IL-10 ratio and IL-8, LDH, and HBDH level predict the severity and the risk of death in AIDS patients with pneumocystis

pneumonia. *J Immunol Res* 2016; 2016: 1583951. DOI: 10.1155/2016/1583951.

26. Apostolov I, Minkov N, Koycheva M, et al. Acute changes of serum markers for tissue damage after ESWL of kidney stones. *Int Urol Nephrol* 1991; 23: 215–220.

27. Campistol JM, Arias M, Ariceta G, et al. An update for atypical haemolytic uraemic syndrome: diagnosis and treatment.

A consensus document. *Nefrologia* 2015; 35: 421–447. DOI: 10.1016/j.nefro.2015.07.005.

28. Hans R, Sharma RR, Marwaha N, et al. Efficacy and safety of therapeutic plasma exchange by using apheresis devices in pediatric atypical hemolytic uremic syndrome patients. *J Clin Apher* 2016; 31: 381–387. DOI: 10.1002/jca.21412.