岩手医科大学
審査学位論文
（博　士）

Ability of a novel system for neonatal extracorporeal renal replacement therapy with an ultra-small volume circuit to remove solutes *in vitro*

Saeko Nishimi · Ken Ishikawa · Makoto Sasaki · Hiromi Furukawa · Akira Takada · Shoichi Chida

Department of Pediatrics, School of Medicine, Iwate Medical University

19-1 Uchimaru, Morioka, Iwate 020-8505, Japan

Telephone +81-19-651-5111

Facsimile +81-19-651-0515

Corresponding author:

Ken Ishikawa,

Department of Pediatrics, School of Medicine, Iwate Medical University

19-1 Uchimaru, Morioka, Iwate 020-8505, Japan

E-mail: kenishi@iwate-med.ac.jp

Abstract

Background We automated our manual, syringe-driven extracorporeal renal replacement therapy (eRRT) system with an ultra-small volume circuit (3.2 mL) suitable for neonates without blood priming.

Objective To determine the solute clearance and water balance of the automated and manual systems *in vitro*.

Methods Stored whole blood samples containing exogenous urea, creatinine (Cr), potassium (K) and ammonia (NH_3) to imitate acute kidney injury and hyperammonemia were dialyzed for three hours (blood flow, 4.0 mL/min; dialysate flow, 600 mL/h) with a continuous infusion of heparin. Then solute clearance and sample weight were compared with values before dialysis.

Results In the manual and automated systems, the median clearance of blood urea nitrogen, Cr, K and NH_3 ranged from 1.7 to 2.3 and from 2.4 to 2.6 mL/min and the median weight of the samples was decreased by 3.8 g and increased by 8.3 g after three hours of dialysis, respectively.

Conclusions The automated system effectively cleared solutes, but safety concerns were associated with platelet consumption and fluid balance. Additional studies are needed to establish the safety and accuracy of this novel system for clinical use in neonates and preterm infants.

Keywords

neonate · hemodialysis · blood priming · single-needle dialysis · automated syringe-driven blood circulation

Introduction

Advances in technology and practical skills for extracorporeal renal replacement therapy (eRRT) have increased the choices of treatment for neonates with acute kidney injury (AKI) or inborn errors of metabolism (IEM) [1, 2]. However, the existing conventional circuit volume for eRRT is excessive compared with the circulating blood volume in neonates (80 ml/kg). Therefore eRRT circuits require blood priming to prevent hemodilution and hemodynamic instability when their extracorporeal circuit volumes are >10% of the circulating blood volume in neonates [3]. Blood priming for circuits can also cause hypotension or an electrolyte imbalance [4, 5] and it is associated with a risk of transfusion-associated complications such as fatal viral infections [6]. Therefore, an eRRT system without blood priming is needed for neonates. We invented a novel manual eRRT system with an ultra-small circuit (total volume, 3.0 mL) and a small hemofilter, which allowed eRRT to proceed in neonates without blood priming. The manual eRRT system was based on single-needle dialysis (SND) [7], and consisted of a small hemofilter, two check valves, a gas-tight syringe, four three-way taps and two tubes. The ability of the manual eRRT system to clear substances associated with AKI and IEM *in vitro* has already been proven [8]. We subsequently automated the manual eRRT system as the first step in the creation of a prototype neonatal eRRT system for clinical applications.

The present study compares the ability of our novel automated and manual eRRT systems with an ultra-small volume circuit to remove solutes and maintain the water balance *in vitro*, with the aim of clinical applications to neonates and preterm infants.

Methods

Circuit design of manual eRRT system

The manual eRRT system comprises a polysulfone hollow fiber dialyzer with a volume of 1.0 mL and a membrane surface area of 0.01 m^2 (Asahi Kasei Medical Co., Tokyo, Japan), two check valves (Kawasumi Laboratories, Inc., Tokyo, Japan), a 2.5-mL gas-tight syringe (Hamilton Co., Reno, NV, USA), four three-way taps that connect the parts but do not alter flow, and two tubes (Fig. 1). The total extracorporeal blood volume of the circuit is 3.0 mL, which is <10% of the circulating blood volume of a neonate weighing 1.0 kg (80 mL). One-way blood circulation is generated by manually pulling and pushing the plunger of the syringe, with two check valves in the circuit. Blood (2.0 mL) drawn from a blood storage bag into the syringe during the pulling phase (Fig. 1a) flows through the dialyzer and is then returned to the blood bag during the pushing phase (Fig. 1b).

Circuit design of automated eRRT system

This circuit comprises the same polysulfone hollow fiber dialyzer with a volume of 1.0 mL and a membrane surface area of 0.01 m^2, a 2.5-mL gas-tight syringe pump with electrical Microlab® PSD/4 Y-valves (Hamilton Co., Reno, NV, USA), one three-way tap that only connects the parts and does not alter the flow, and two tubes (Fig. 2). The total extracorporeal blood volume of the circuit is 3.2 mL. One-way blood circulation is generated through the electric Y-valve in the syringe pump by automatically pulling and pushing the plunger of the syringe. Blood (2.0 mL) drawn from a blood storage bag during the pulling phase flows through the dialyzer into the syringe (Fig. 2a) and then returns to the bag during the pushing phase (Fig. 2b).

Assessment of solute clearance

Whole blood samples (100 mL) collected from 16 adult volunteers were stored in separate storage bags with heparin (100 units/bag). Powdered urea and creatinine, a solution of potassium L-aspartate and aqueous ammonia were added to the bags to imitate the clinical conditions associated with AKI or IEM. The concentration of added urea was measured as blood urea nitrogen (BUN). The concentrations of each of these additive solutes were such that they increased levels of BUN, creatinine (Cr), potassium (K) and ammonia (NH_3) to ≥80 mg/dL, ≥4.0 mg/dL, ≥8.0 mEq/L and ≥600 μg/dL, respectively.

Informed consent was obtained from all participating volunteers, and this study was approved by Independent Ethics Committee in Iwate Medical University (approval number: H22-33).

Experimental protocol

The manual and automated eRRT circuits were washed with heparinized (heparin 10 units/mL) saline and then connected to a blood storage bag using an 18-gauge intravenous SURFLO® catheter (Terumo Co., Tokyo, Japan). Sixteen blood specimens (n = 8 each for the manual and automated systems) were dialyzed for three hours. The elapsed time of the syringe stroke phase was 15 sec, and the blood flowed at a rate equivalent to 4.0 mL/min, which corresponded to a clinical rate of 3.0 – 4.0 mL/kg per min [9]. We used the SUBPACK-Bi® dialysate (NIPRO Corp., Osaka, Japan) containing Na, 140 mEq/L; K, 2.0 mEq/L; Cl, 113 mEq/L; Ca^{2+}, 3.5 mEq/L; HCO_3^-, 35 mEq/L.

The conventional Plasouto iQ21® eRRT device (Asahi Kasei Medical Co., Tokyo, Japan) generated dialysate flow in the opposite direction to that of the

blood at flow rate of 600 mL/h, which was 2.5 times of the blood flow rate, because solute clearance gradually peaks under such conditions. Fluid was deliberately not removed during these experiments. A continuous infusion of heparin (30 units/h) delivered using an ordinary infusion pump via a three-way tap in the manual system and directly to the blood bag in the automated system provided anticoagulation, which resulted in an approximate activated clotting time (ACT) of 300 sec.

Measured parameters

Complete counts of red blood cells, white blood cells, as well as levels of hemoglobin, hematocrit, platelets, total protein, albumin, total bilirubin, aspartate aminotransferase, lactate dehydrogenase, BUN, Cr, Na, K, Cl, Ca^{2+}, lactic acid, NH_3, glucose, HCO_3^- and ACT were assessed before and after three hours of dialysis. Blood (2.0 mL) directly withdrawn from the blood storage bag was tested before dialysis and after starting dialysis. Solute clearance and reduction rates were calculated for BUN, Cr, K, and NH_3 as $(C_{pre} - C_{post})/C_{pre}$*Qb and $(C_{pre} - C_{post})/C_{pre}$, respectively, where C_{pre} and C_{post} represent concentrations of the solutes before and after three hours of dialysis and Qb represents blood flow. The blood storage bags were weighed every 30 minutes to assess the accuracy of the water balance. Pressure in the circuit, such as the transmembrane pressure (TMP), was not recorded. The eRRT circuits were disconnected after the experiments and blood clot formation was assessed.

Statistical analysis

Values before and after three hours of dialysis were compared using the Wilcoxon signed-rank test. Values were compared between the manual and automated

systems using the Mann-Whitney rank sum test. All data were analyzed using SigmaPlot (Systat Software, San Jose, CA, USA). P values <0.05 were considered statistically significant. All data are presented as medians (range).

Results

The clearance of BUN, Cr, K, and NH_3 after three hours of dialysis in the manual system (Fig. 3) was 2.3 (1.9 – 2.6), 2.3 (2.2 – 2.6), 2.2 (2.0 – 2.6) and 1.7 (-0.4 – 1.9) mL/min, respectively, resulting in reduction rates of 58.5% (48.0% – 66.1%), 58.1% (54.7% – 65.9%), 54.6% (49.1% – 64.0%), and 42.4% (11.1% – 64.6%), respectively. In the automated system, the clearance of BUN, Cr, K and NH_3 after three hours of dialysis (Fig. 4) was 2.5 (2.3 – 3.7), 2.6 (2.4 – 3.5), 2.5 (2.3 – 3.3) and 2.4 (1.8 – 3.2) mL/min, respectively, resulting in reduction rates of 62.4% (56.7% – 92.1%), 65.9% (59.4% – 87.2%), 61.5% (56.6% – 81.5%), and 58.8% (43.9% – 79.7%), respectively. The clearances of Cr ($p < 0.05$), K ($p < 0.05$), and NH_3 ($p < 0.01$) were significantly higher in the automated, than in the manual system.

The platelet count significantly decreased, whereas aspartate aminotransferase, lactate dehydrogenase, Ca^{2+}, lactic acid and HCO_3^- values significantly increased after three hours of dialysis in the manual system (Table 1). In the automated system, values for red blood cells, hemoglobin, hematocrit, white blood cells, platelets, total protein, albumin and glucose significantly decreased, whereas those for Ca^{2+} and HCO_3^- significantly increased after three hours of dialysis (Table 1).

The weight of the blood storage bags did not statistically change in the manual system, but significantly increased by 8.3 (2.0 – 31.5) g ($p < 0.01$) in the automated system after three hours of dialysis (Fig. 5). One experiment in the

automated system stopped at 2.5 hours of dialysis, because a negative transmembrane pressure (TMP) automatically ceased work of the conventional eRRT device. The negative TMP did not reflect true value, and showed inaccurate pressure of dialysate-out without pressure of dialysate-in and inlet and outlet of blood flow, because we used the conventional eRRT device to control only flow of dialysate and effluent.

Tiny clots occasionally formed after the experiments at the three-way taps, check valves, and syringe plunger of the manual system, and in the tubes of the automated system.

Discussion

We upgraded a manual eRRT system with an ultra-small circuit volume (3.0 mL) based on SND [7] into an automated eRRT system that does not require blood priming. This could therefore be of clinical benefit to neonates and preterm infants. Here, we described our concept of manual and automated neonatal eRRT systems and compared their abilities to remove solutes and maintain water balance during hemodialysis for three hours *in vitro*. The automated eRRT system more efficiently cleared BUN, Cr, K, and NH_3 solutes, which would be increased in AKI and/or IEM.

Two eRRT systems have been specifically developed for small infants and neonates. The conventional Cardio-Renal Pediatric Dialysis Emergency Machine (CA.R.PE.DI.E.M) [10] uses a roller pump for blood circulation and double-lumen vascular access for inflow and outflow, which reduces the extracorporeal circuit volume to 27.2 mL. However, this eRRT system requires blood priming to

prevent hemodilution and hemodynamic instability, even for neonates weighing around 3.0 kg in which the circulating blood volume would be about 240 mL.

A novel system based on the SND system developed by Coulthard et al. [11] and Everdell et al. [12] requires only one blood access route for alternating inflow into and outflow from the blood circulation [7], which reduces circuit volumes. The eRRT circuit volumes based on the SND system for children have been reduced by using two syringes to drive the blood circulation [11-14]. In these systems blood is drawn from a patient into syringe 1 and sent through the hemofilter to syringe 2. Blood is pushed back and forth between the two syringes until sufficient solutes and volume have been removed, and the remainder is returned to the patient. The syringe-driven SND system developed by Coulthard et al. was called Nidus (the Newcastle infant dialysis and ultrafiltration system) [11]. It has a significantly minimal operating circuit volume of 9.3 mL for clinical use and eRRT can theoretically proceed without blood priming on neonates weighing >1,200 g with a circulating blood volume of >96 mL. This system has been applied to neonates and infants weighing 1.8 – 7.0 kg [11].

De Virgiliis et al. [15] reported a one syringe-driven SND system for adult patients that generates one-way blood circulation by alternately pulling and pushing the plunger of the syringe while automatically opening and closing each of two clamps. This SND system sufficiently clears solutes from adult patients. Our eRRT system resembles this. Our automated eRRT system comprises a small-volume (1.0 mL) hemofilter with a surface area of 0.01 m^2 and one syringe pump with electrical Y-valves, which reduces the entire extracorporeal circuit volume to 3.2 mL (Fig. 2). One-way blood circulation is simply generated by the electrical Y-valve in the syringe pump via automatically pulling and pushing the syringe

plunger. Our automated system can theoretically perform eRRT on neonates, including extremely low birth weight infants weighing >400 g with a circulating blood volume of >32 mL without the need for blood priming.

Concentrations of Cr, K, and NH_3 were reduced more effectively by the automated rather than the manual system. The clearance rate of the conventional CA.R.PE.DI.E.M eRRT system for infants is 2.5 mL/min and the surface area of the dialyzer is 0.075 m^2 [10]. Everdell et al. treated four infants weighing 0.8 – 3.84 kg using the SND system, which resulted in BUN, Cr and K clearance rates of about 0.5 mL/min, with a blood flow rate of 2.0 – 20 mL/min and a dialyzer with a surface area of 0.042 m^2 [12]. Coulthard et al. reported BUN, Cr and phosphate clearance rates of around 1.5 – 2.0 mL/min using the Nidus system with a blood flow rate of 5.0 mL/min and a dialyzer with a surface area of 0.045 m^2 [11]. Although the present assessment of our automated system proceeded *in vitro*, it should sufficiently clear solutes for clinical use in neonates because small solute removal is mostly determined by the dialysate/ultrafiltration flow rate rather than the hemofilter surface area [16].

Our automated system has serious weaknesses that will require resolution. First being that water balance was not accurately maintained. Precise water balance is very important during eRRT for neonates because large errors could cause volume overload, hemodilution, or hemodynamic instability. The syringe pump in the automated system was positioned after the hemofilter (Fig. 2), which might draw dialysate into the blood circulation by negative pressure on the hemofilter during the pulling phase. The drawn dialysate might thus cause hemodilution of the blood specimens and decrease hemoglobin, hematocrit, total protein, and albumin. In contrast, the syringe in the manual system is placed in

front of the hemofilter, which might avoid applying negative pressure on the hemofilter during the pulling phase. The optimal position of the syringe should be assessed. The accuracy of water balance was very precisely controlled in the Nidus system compared with a conventional pediatric hemodialysis machine [11]. The accuracy of ultrafiltration in the CA.R.PE.DI.E.M system always remained within the limit of 1 g/h and variations associated with different transmembrane pressures and filtration rates were insignificant [17]. Therefore a dedicated device is needed to precisely control ultrafiltration and dialysate flow in the automated system to ensure accurate ultrafiltration.

The platelet count decreased after three hours of hemodialysis in both eRRT systems, perhaps because of platelet activation and degranulation, which might be due to blood becoming exposed to the roller pump segment of the dialysis tubing, microbubbles, or dialysate [18]. Platelet activation and aggregation have been found during dialysis with polysulfone membranes in dialyzers sterilized by an electron beam [19]. We applied to maintain ACT at 300 sec in this study, because clots developed in the first pilot study of the manual system when ACT was maintained at 200 sec. However, tiny clots occasionally developed at the three-way taps, check valves, syringe plunger, or in the tubes; perhaps as a result of accelerated platelet aggregation. Moreover, Mulder et al. associated a low blood flow rate during eRRT with thrombocytopenia due to either the destruction or retention of platelets during passage through the hemofilter [20]. In addition, LDH level increased in the manual system, which might be explained by the release of LDH from platelets adhering to the hemofilter membrane [18]. Also the increased levels of LDH in the manual system might be due to hemolysis or blood aggregation caused by irregular movements of the syringe, since such increases were not evident in the automated system. Thus, the nature of the material in the

hemofilter together with the hemofilter shape that would be optimal for low blood-flow rate in our eRRT system should be examined. In addition, we will investigate the ability of nafamostat mesilate to attenuate platelet aggregation, as this is the main anticoagulation drug used for eRRT in Japan.

Lactic acid concentrations significantly increased after three hours of dialysis in the manual system, but did not significantly change in the automated system, which could be affected by hemodilution following weight increases. However, lactic acid was also increased in whole blood in storage bags held for three hours; as such, this might be associated with anaerobic conditions *in vitro*. Further investigation is needed to confirm whether anaerobic conditions are also induced by the automated eRRT system *in vivo* before it could be clinically applied to neonates and preterm infants with low birth weight.

Our automated eRRT system is presently under further development for clinical use in neonates. We are working to refine the system to achieve necessary improvements from the perspectives of heat conservation, accurate water balance, monitoring of pressure in the circuit, detection of air, and the size and shape of the dialyzer, before clinical application to neonates can be performed.

Conclusions

Our automated eRRT system using an ultra-small volume circuit can remove accumulated solutes associated with AKI and IEM *in vitro*. Although this system could be applicable even to neonates without blood priming, safety concerns related to platelet consumption and fluid balance need to be addressed. Additional studies are needed to establish the safety and accuracy of this novel eRRT system for clinical use in neonates.

Acknowledgements

We are grateful to the Mother and Child Health Foundation (Grant No. 23-9). We also thank Asahi Kasei Medical Co., Ltd. for supplying the small hemofilters.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Gouyon JB, Guignard JP (2000) Management of acute renal failure in newborns. Pediatr Nephrol 14:1037–1044

2. Fleming GM, Walters S, Goldstein SL, Alexander SR, Baum MA, Blowey DL, Bunchman TE, Chua AN, Fletcher SA, Flores FX, Fortenberry JD, Hackbarth R, McBryde K, Somers MJG, Symons JM, Brophy PD (2012) Nonrenal indications for continuous renal replacement therapy: a report from the Prospective Pediatric Continuous Renal Replacement Therapy Registry Group. Pediatr Crit Care Med 13:e299–304

3. Goldstein SL (2011) Advances in pediatric renal replacement therapy for acute kidney injury. Semin Dial 24:187–191

4. Santiago MJ, López-Herce J, Urbano J, Solana MJ, del Castillo J, Ballestero Y, Botrán M, Bellón JM (2009) Complications of continuous renal replacement therapy in critically ill children: a prospective observational evaluation study. Crit Care 13:R184

5. Hackbarth RM, Eding D, Gianoli Smith C, Koch A, Sanfilippo DJ, Bunchman TE (2005) Zero balance ultrafiltration (Z-BUF) in blood-primed CRRT circuits achieves electrolyte and acid-base homeostasis prior to patient connection. Pediatr Nephrol 20:1328–1333

6. Secher EL, Stensballe J, Afshari A (2013) Transfusion in critically ill children:

an ongoing dilemma. Acta Anaesthesiol Scand 57:684–691

7. Kopp KF, Gutch CF, Kolff WJ (1972) Single needle dialysis. Trans Am Soc Artif Intern Organs 18:75–81

8. Nishimi S, Ishikawa K, Oda S, Furukawa H, Takada A, Chida S (2015) *In vitro* ability of a novel system for neonatal extracorporeal renal replacement therapy with ultra-small volume circuit for removing solutes. JIMA (in press)

9. Bunchman TE, Donckerwolcke RA (1994) Continuous arterial-venous diahemofiltration and continuous veno-venous diahemofiltration in infants and children. Pediatr Nephrol 8: 96–102

10. Ronco C, Garzotto F, Ricci Z (2012) CA.R.PE.DI.E.M (Cardio-Renal Pediatric Dialysis Emergency Machine): evolution of continuous renal replacement therapies in infants. A personal journey. Pediatr Nephrol 27:1203–1211

11. Coulthard MG, Crosier J, Griffiths C, Smith J, Drinnan M, Whitaker M, Beckwith R, Matthews JNS, Flecknell P, Lambert HJ (2014) Haemodialysing babies weighing <8 kg with the Newcastle infant dialysis and ultrafiltration system (Nidus): comparison with peritoneal and conventional haemodialysis. Pediatr Nephrol 29:1873–1881

12. Everdell NL, Coulthard MG, Crosier J, Keir MJ (2005) A machine for haemodialysing very small infants. Pediatr Nephrol 20:636–643

13. Everdell NL (2007) A haemodialysis system for the treatment of acute renal failure and metabolic disorders in neonates. Med Eng Phys 29:516–524

14. Coulthard MG, Sharp J (1995) Haemodialysis and ultrafiltration in babies weighing under 1000 g. Arch Dis Child Fetal Neonatal Ed 73:F162–165

15. De Virgiliis G, Vanin M, Buoncristiani U (1985) A new single needle dialysis system. Trans Am Soc Artif Intern Organs 31:116–118

16. Murray PT, Brady HR, Hall JB (2006) Intensive care in nephrology. Taylor & Francis, UK, p 204

17. Ronco C, Garzotto F, Brendolan A, Zanella M, Bellettato M, Vedovato S, Chiarenza F, Ricci Z, Goldstein SL (2014) Continuous renal replacement therapy in neonates and small infants: development and first-in-human use of a miniaturised machine (CA.R.PE.DI.E.M). Lancet 383:1807–1813

18. Daugirdas JT, Bernardo AA (2012) Hemodialysis effect on platelet count and function and hemodialysis-associated thrombocytopenia. Kidney Int 82:147–157

19. Kiaii M, Djurdjev O, Farah M, Levin A, Jung B, MacRae J (2011) Use of electron-beam sterilized hemodialysis membranes and risk of thrombocytopenia. JAMA 306:1679–1687

20. Mulder J, Tan HK, Bellomo R, Silvester W (2003) Platelet loss across the hemofilter during continuous hemofiltration. Int J Artif Organs 26:906–912

Figure legends

Fig. 1. Schematic diagram and picture of the manual extracorporeal renal replacement therapy systems with ultra-small volume circuit.

Blood is manually withdrawn from blood storage bag into a syringe (a, solid line), then returned to bag through dialyzer (b, dashed line).

Fig. 2. Schematic diagram and picture of the automated extracorporeal renal replacement therapy system with ultra-small volume circuit.

Blood is automatically withdrawn from blood storage bag through the dialyzer into a syringe during the pulling phase (a, solid line) and then returned to the bag during the pushing phase (b, dashed line).

Fig. 3. Comparison of solute concentrations before and after three hours of dialysis in the manual system.

(a) Blood urea nitrogen, (b) creatinine, (c) potassium, and (d) ammonia.

Lines from the bottom line to the top of the box plots represent minimum values, first quartile points, medians, third quartile point and maximum values ($n = 8$; $^{*}p < 0.05$, $^{\dagger}p < 0.01$).

Fig. 4. Comparison of solute concentrations before and after three hours of dialysis in the automated system.

(a) Blood urea nitrogen, (b) creatinine, (c) potassium, and (d) ammonia.

Lines from the bottom line to the top of the box plots represent minimum values, first quartile points, medians, third quartile point and maximum values ($n = 8$; $^{\dagger}p < 0.01$).

Fig. 5. Weight of blood storage bags in the manual and automated systems every half hour after starting dialysis.

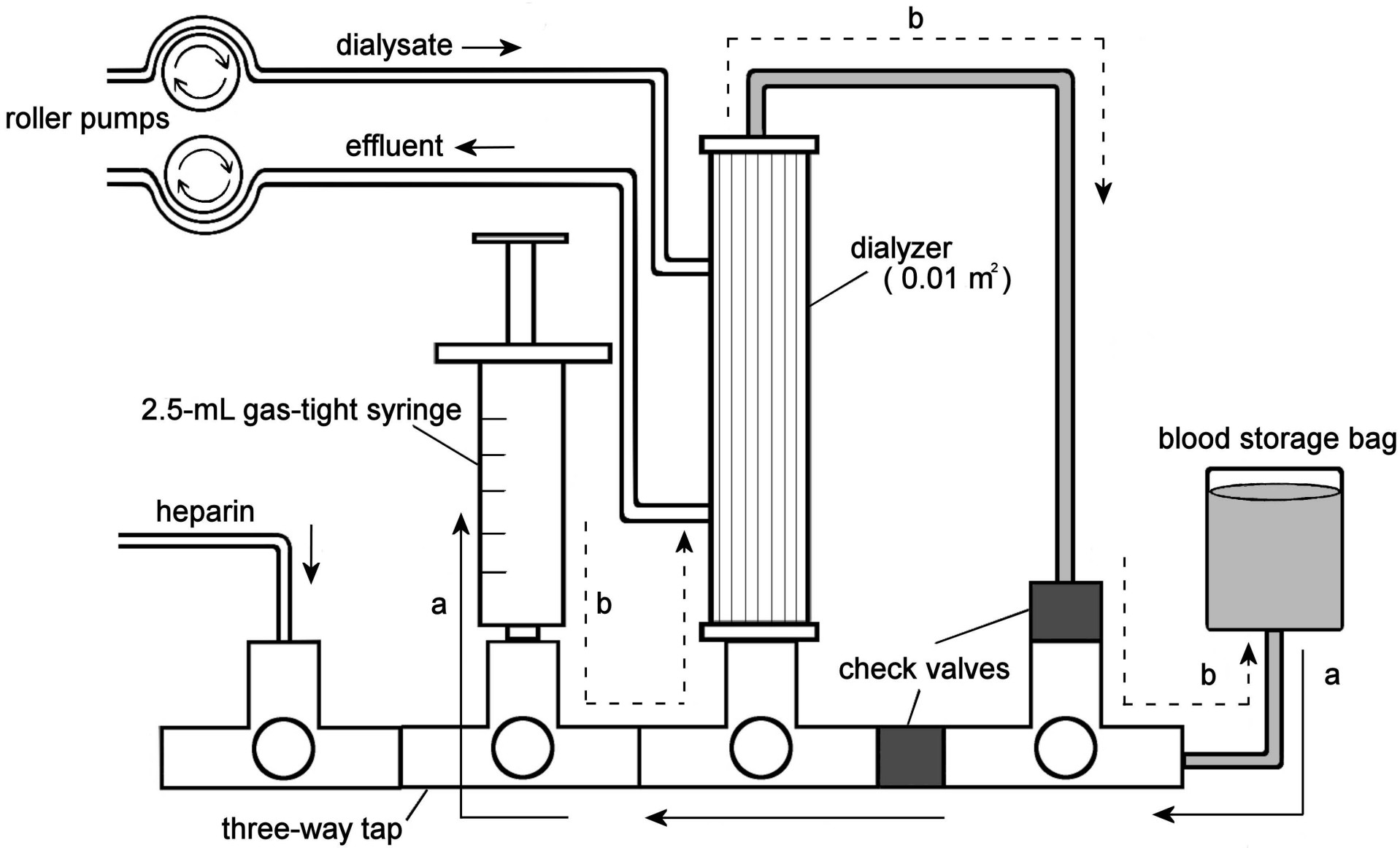
(a) Manual (solid line) and (b) automated (dashed line) systems ($n = 8$ each).

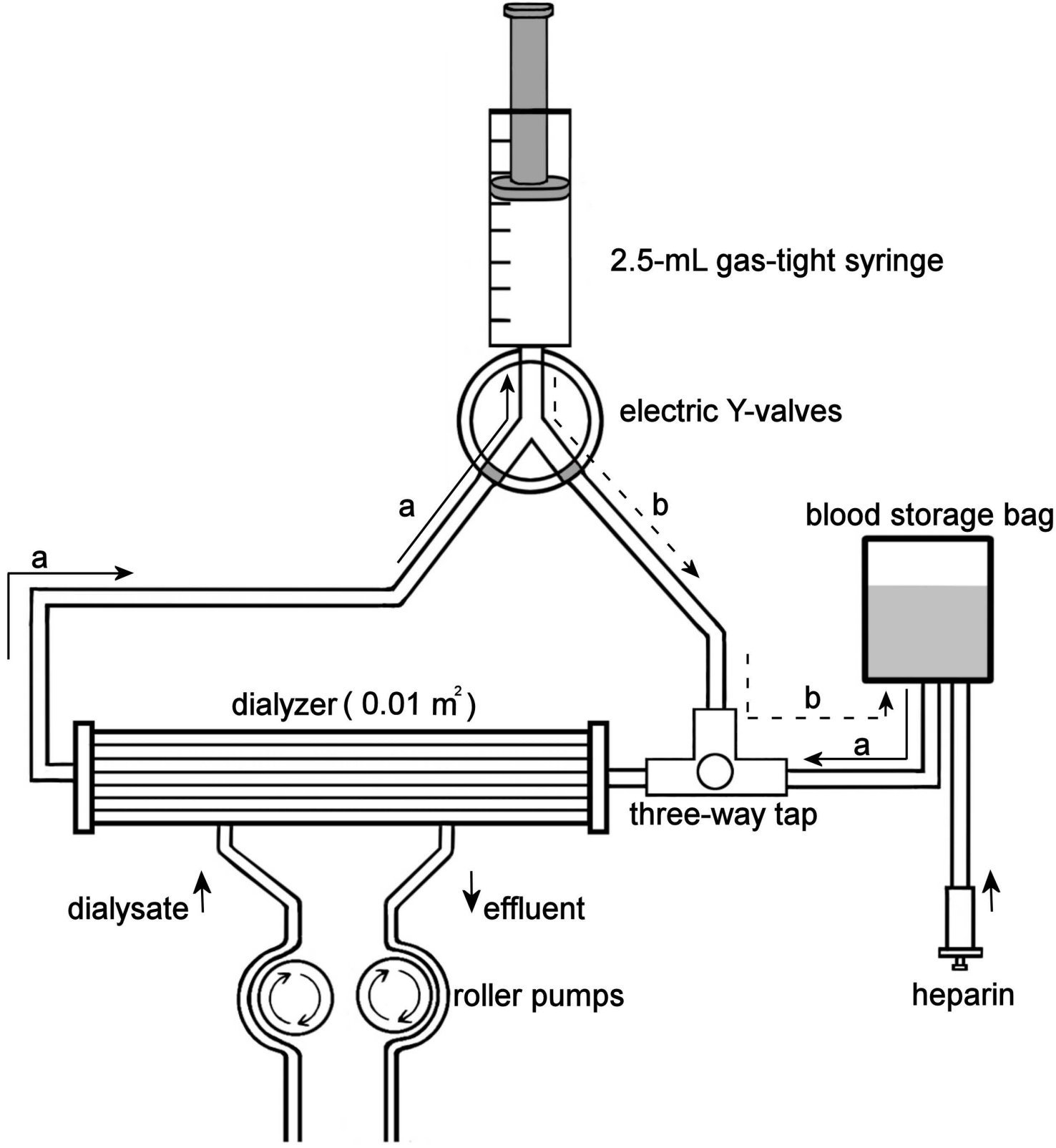
Infusion volume of heparin was subtracted from the weight of each bag.

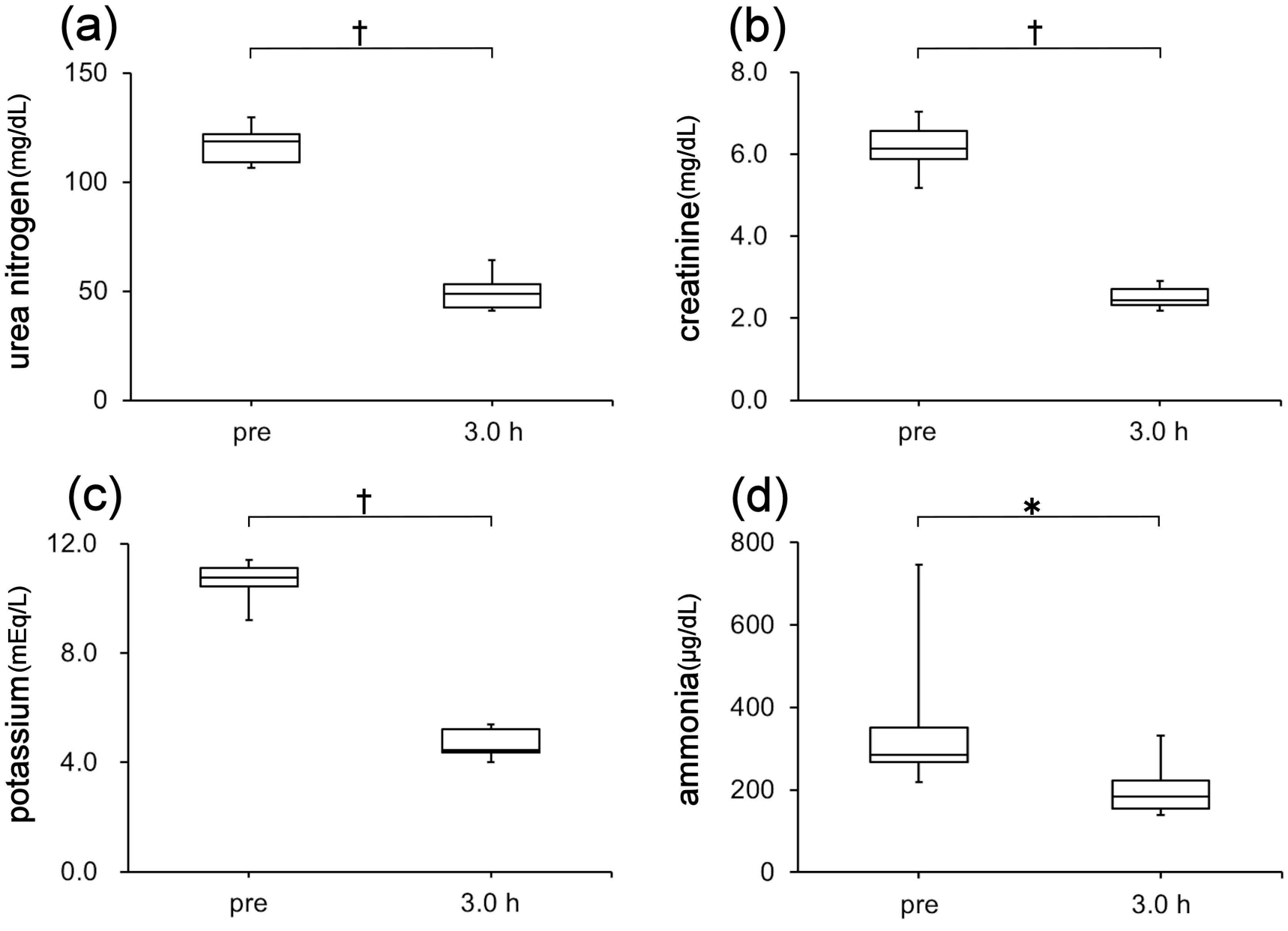
Table 1 Concentrations of measured parameters before and after three hours of dialysis in the manual and automated systems

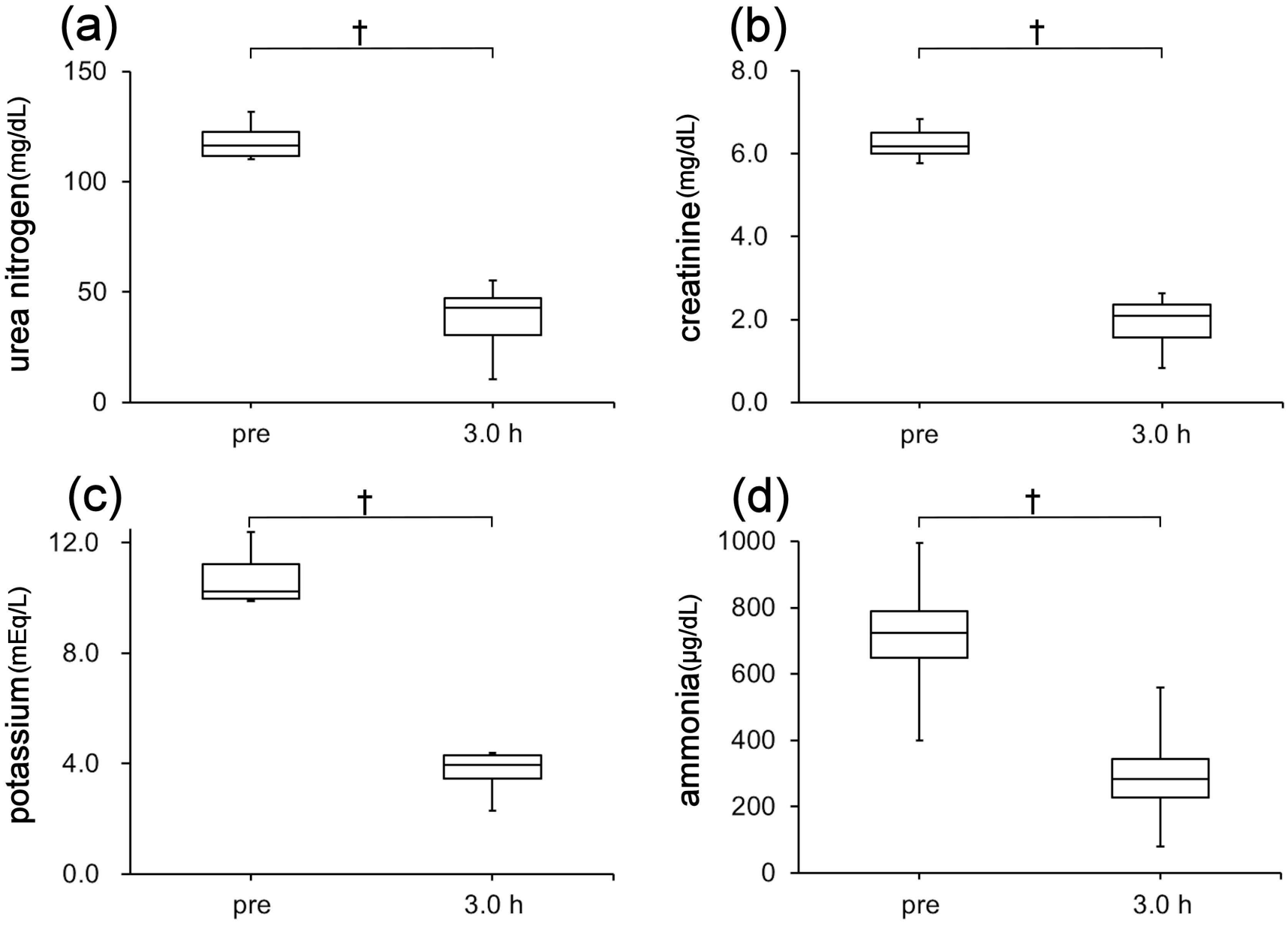
	manual system			automated system		
	predialysis	postdialysis	*p*	predialysis	postdialysis	*p*
Red blood cells ($\times 10^6$/μL)	4.5 (4.0–4.7)	4.6 (3.9–5.2)	ns	4.3 (4.0–5.1)	3.7 (2.8–4.6)	< 0.01
Hemogrobin (g/dL)	13.9 (11.6–14.6)	14.3 (11.5–15.5)	ns	12.6 (11.5–14.2)	10.4 (8.0–13.0)	< 0.01
Hematocrit (%)	42.4 (35.4–44.2)	43.9 (35.7–50.2)	ns	38.7 (34.6–45.6)	32.8 (25.1–41.4)	< 0.01
White blood cells ($\times 10^3$/μL)	5.1 (3.9–8.04)	5.1 (3.5–7.08)	ns	5.4 (4.4–7.3)	4.4 (3.6–6.4)	< 0.01
Platelets ($\times 10^3$/μL)	222 (162–288)	176 (135–244)	< 0.05	289 (193–305)	219 (164–259)	< 0.01
Total protein (g/dL)	7.0 (6.7–7.4)	7.7 (6.5–9.4)	ns	7.0 (6.6–7.3)	6.0 (4.5–6.7)	< 0.01
Albumin (g/dL)	4.6 (1.82–5.01)	4.9 (3.68–6.21)	ns	4.6 (4.4–4.8)	3.8 (3.0–4.3)	< 0.01
Total bilirubin (mg/dL)	0.4 (0.0–0.7)	0.3 (0.0–1.0)	ns	0.0 (0.0–0.5)	0.1 (0.0-0.4)	ns
Aspartate aminotransferase (IU/L)	18 (15–22)	34 (28–42)	< 0.01	16 (14–89)	15 (12–84)	ns
Lactate dehydrogenase (IU/L)	172 (133–201)	399 (296–483)	< 0.01	171 (131–253)	196 (121–485)	ns
Urea nitrogen (mg/dL)	118.5 (106.3–129.5)	48.8 (41.1–64.3)	< 0.01	116.5 (110.2–131.6)	43.0 (10.4–55.3)	< 0.01
Creatinine (mg/dL)	6.1 (5.2–7.0)	2.4 (2.2–2.9)	< 0.01	6.2 (5.8–6.8)	2.1 (0.8–2.6)	< 0.01
Na (mEql/L)	135 (133–137)	136 (134–138)	ns	136 (134–139)	137 (135–138)	ns
K (mEq/L)	10.8 (9.2–11.4)	4.5 (4.0–5.4)	< 0.01	10.3 (9.9–12.4)	4.0 (2.3–4.4)	< 0.01
Cl (mEq/L)	106 (103–111)	104 (101–106)	< 0.05	107 (104–109)	105 (104–107)	< 0.05
Ca^{2+} (mmol/L)	1.1 (1.1–1.18)	1.4 (1.3–1.4)	< 0.01	1.1 (1.1–1.2)	1.4 (1.3–1.5)	< 0.01
Lactic acid (mmol/L)	1.3 (0.8–1.8)	2.0 (1.6–2.6)	< 0.01	1.6 (1.2–2.0)	1.7 (1.0–2.5)	ns
Ammonia (μg/dL)	286 (219–746)	183 (140–331)	< 0.05	724 (399–995)	284 (81–558)	< 0.01
Glucose (mg/dL)	92 (63–114)	80 (75–85)	ns	88 (80–95)	78 (69–85)	< 0.01
$HC0_3^-$ (mmol/L)	24.5 (22.8–25.6)	26.5 (24.9–27.4)	< 0.01	23.3 (21–26.5)	27.2 (24.7–27.9)	< 0.01
Activated clotting time (sec)	318 (246–396)	382 (286–440)	ns	365 (298–800)	372 (303–474)	ns

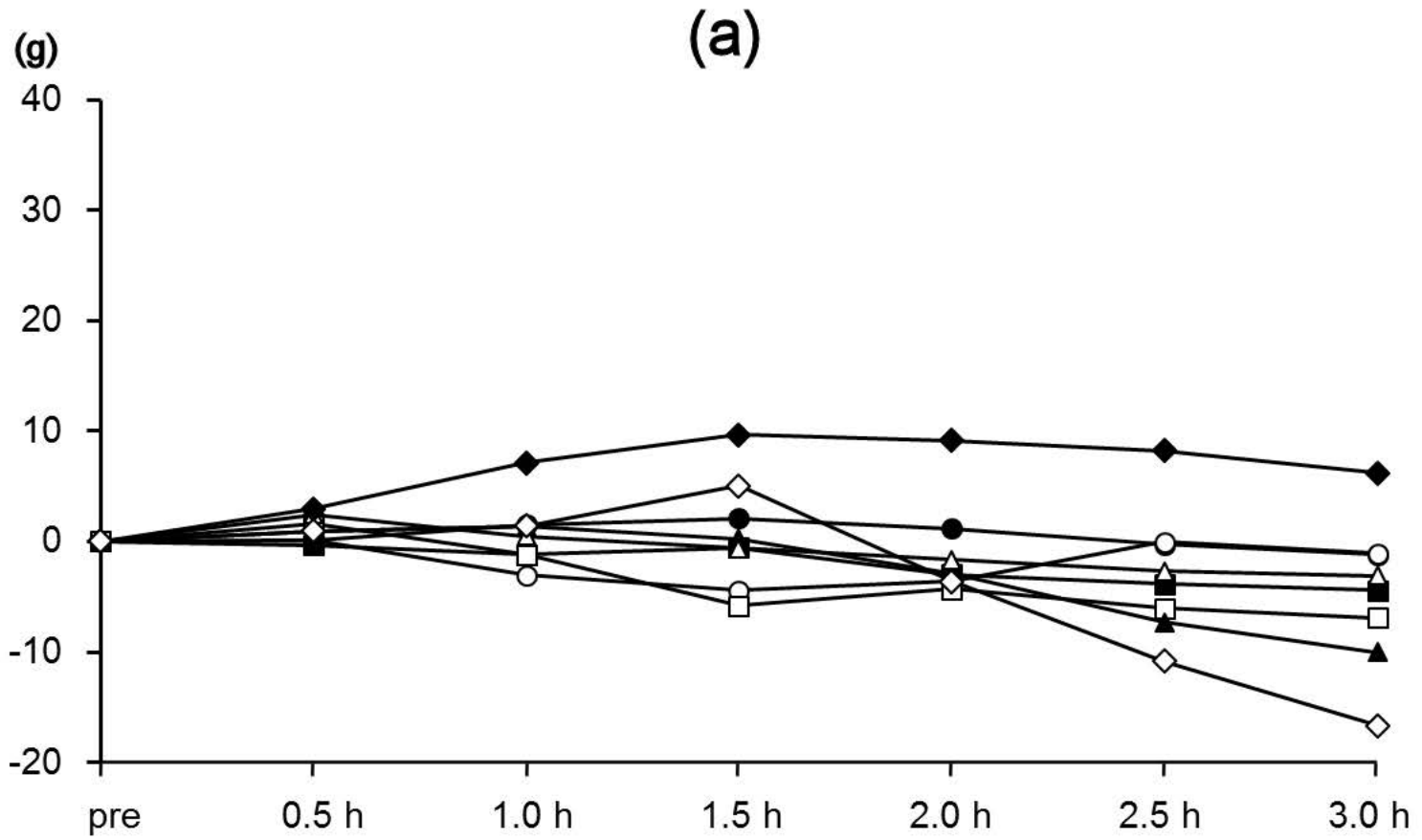
Values indicate medians (range). *ns* not significant

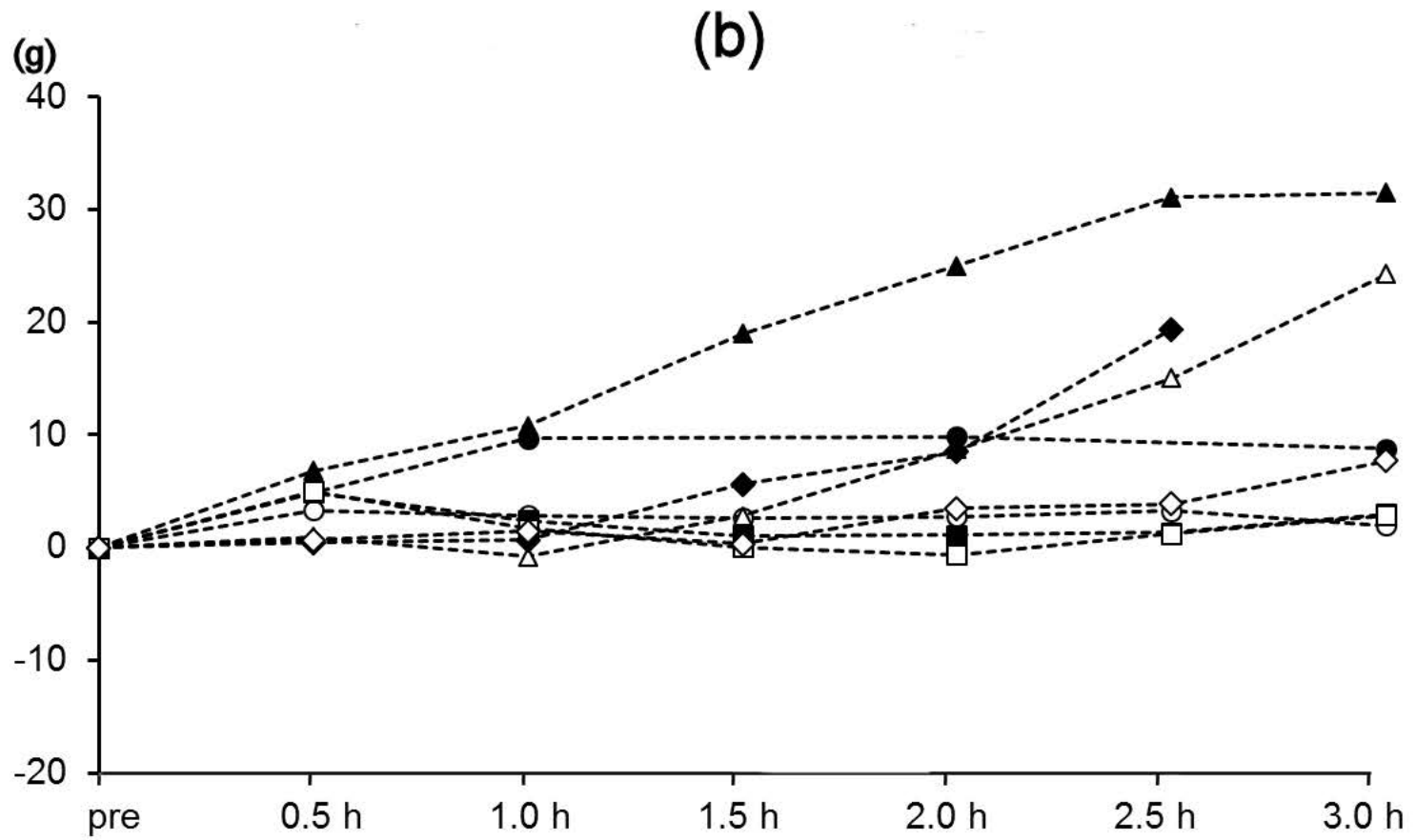

b
dialysate
roller pumps
effluent
dialyzer
(0.01 m²)
2.5-mL gas-tight syringe
blood storage bag
heparin
a
b
check valves
b
a
three-way tap


2.5-mL gas-tight syringe
electric Y-valves
a
b
blood storage bag
a
dialyzer (0.01 m^2)
b
a
three-way tap
dialysate
effluent
roller pumps
heparin


(a)
†
150
100
50
0
urea nitrogen(mg/dL)
pre
3.0 h
(b)
†
8.0
6.0
4.0
2.0
0.0
creatinine(mg/dL)
pre
3.0 h
(c)
†
12.0
8.0
4.0
0.0
potassium(mEq/L)
pre
3.0 h
(d)
*
800
600
400
200
0
ammonia(μg/dL)
pre
3.0 h


(a)
†
150
100
50
0
urea nitrogen(mg/dL)
pre
3.0 h
(b)
†
8.0
6.0
4.0
2.0
0.0
creatinine(mg/dL)
pre
3.0 h
(c)
†
12.0
8.0
4.0
0.0
potassium(mEq/L)
pre
3.0 h
(d)
†
1000
800
600
400
200
0
ammonia(μg/dL)
pre
3.0 h


(a)
(g)
40
30
20
10
0
-10
-20
pre
0.5 h
1.0 h
1.5 h
2.0 h
2.5 h
3.0 h


(b)
(g)
40
30
20
10
0
-10
-20
pre
0.5 h
1.0 h
1.5 h
2.0 h
2.5 h
3.0 h