

Original Article

Trace element removal during *in vitro* and *in vivo* continuous haemodialysis

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Abstract

**Background.** Continuous renal replacement therapy (CRRT) increasingly is being used to treat critically ill patients with renal disease. CRRT removes waste products but also nutrients. Our understanding of trace element CRRT clearance has been limited by poor assay sensitivity. The development of inductively coupled plasma mass spectrometry (ICP-MS) allows for the measurement of CRRT trace element removal.

**Methods.** Continuous venovenous haemodialysis (CVVHD) transmembrane clearances of trace elements and urea were assessed using a bovine blood-based *in vitro* model using two different haemodialyser types. These findings were validated in 10 critically ill adult patients receiving continuous venovenous haemodiafiltration (CVVHDF). Calculated daily trace element loss was compared with a typical dose of daily trace element supplementation.

**Results.** The mean  $\pm$  SD *in vitro* CVVHD transmembrane clearances (ml/min) for the polysulfone haemodialyser were chromium  $0.97 \pm 0.23$ , copper  $0.47 \pm 0.18$ , manganese  $4.6 \pm 3.6$ , selenium  $1.2 \pm 0.63$  and zinc  $2.3 \pm 0.32$  and for the cellulose diacetate haemodialyser chromium  $1.54 \pm 0.91$ , copper  $0.21 \pm 0.07$ , manganese  $7.8 \pm 4.1$ , selenium  $0.76 \pm 0.39$  and zinc  $2.7 \pm 0.37$ . The *in vivo* CVVHDF transmembrane clearances (ml/min) were chromium  $5.4 \pm 2.4$ , copper  $0.45 \pm 0.33$ , manganese  $1.9 \pm 4.6$ , selenium  $1.6 \pm 1.2$ , and zinc  $4.0 \pm 1.3$ .

**Conclusion.** ICP-MS assays detected the five trace elements in the effluent of CVVHDF patients. Trace element CVVHD transmembrane clearance estimates for our *in vitro* model were supported by the *in vivo* CVVHDF findings. Calculated daily trace element loss attributed to CVVHD and CVVHDF with dialysate

flow rates of 33.3 ml/min is less than what is provided in a daily dose of a trace element supplementation product.

**Keywords:** continuous dialysis; continuous renal replacement therapy; critically ill; haemodiafiltration; nutrition; trace elements

Introduction

Acute renal failure (ARF) occurs in approximately 2–5% of hospitalized patients [1] and in 5–6% of critically ill patients [2]. Critically ill ARF patients are often treated with continuous renal replacement therapy (CRRT). Although CRRT removes waste products, it also removes beneficial medications and nutrients. Limited information is available on CRRT drug removal [3] but even less is known about nutrient clearance during CRRT, particularly the CRRT clearance of trace elements.

Available data on trace element abnormalities in renal failure patients have been generally derived from chronic renal failure patients. Extrapolation of these data to ARF patients is difficult especially for patients treated with CRRT. Information on optimal trace element requirements in patients receiving CRRT is scarce [4]. Bellomo [5] proposed that the recommended dietary allowances (RDA) of all trace elements be provided and Druml [6], in a CRRT nutrition review suggested providing trace element supplementation twice weekly. Currently, there is little evidence to support either suggestion. Story *et al.* [7] reviewed the potential loss of chromium, copper, manganese, selenium and zinc in critically ill patients receiving continuous venovenous haemofiltration (CVVH). Chromium and copper were found in the ultrafiltrate of CVVH patients. Critically ill patients had lower blood concentrations of selenium and zinc whether or not they were receiving CVVH when compared with

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normal healthy volunteers. Klein *et al.* [8] detected a small amount of zinc in the effluent of both their CVVH and continuous venovenous haemodialysis (CVVHD) patients. The authors concluded that zinc losses were not large enough to merit additional zinc supplementation in patients treated with CRRT who are receiving zinc in their parenteral nutrition. Berger *et al.* [9] reported that copper, selenium and zinc were found in the effluent during treatment with continuous venovenous haemodiafiltration (CVVHDF). Their most significant finding was that selenium was removed by CVVHDF at two times the daily supplementation.

Since Story *et al.* [7] published their findings, more sensitive trace element assay techniques have been developed. Inductively coupled plasma mass spectrometry (ICP-MS) can detect 90% of the periodic table at the sub-nanogram per gram level [10]. Utilizing this assay, we conducted an *in vitro* CVVH trace element clearance study [11]. Our findings reported measurable quantities of chromium, copper, manganese, selenium, and zinc in the ultrafiltrate of this *in vitro* CRRT model. Trace element loss was estimated using study-derived sieving coefficients (SC) and normal trace element blood concentrations. Our findings suggested that the removal rate of selenium by CVVH was greater than typical supplementation.

These findings led us to further investigate the potential *in vivo* loss of trace elements during other forms of CRRT since the removal characteristics of CVVHD or CVVHDF differ from CVVH. Further, we wished to determine whether our *in vitro* CRRT models were representative of trace element removal in critically ill patients receiving CRRT. We began this investigation by determining the transmembrane clearance of selected trace elements with our *in vitro* CVVHD model. We then followed up the *in vitro* data; by determining trace element transmembrane clearance in 10 critically ill patients treated with CVVHDF and compared these findings to our *in vitro* transmembrane clearance data.

## Materials and methods

### *In vitro* CVVHD

The trace element transmembrane clearance by common CRRT techniques was assessed using an *in vitro* bovine blood CRRT model developed in our laboratory [11–14]. The CVVHD model used 500 ml of pH regulated, continuously

stirred bovine blood anticoagulated with 3.8% sodium citrate. This blood was placed in a 1 l Erlenmeyer flask which was submerged in a 37°C water bath.

Urea (Sigma, St. Louis, MO, USA lot #30K0221) was added to the bovine blood as a control solute to yield initial blood urea nitrogen (BUN) concentration of 75 mg/dl. B. Braun Diapact® CRRT machines and B. Braun Diapact CRRT™ tubing kits for the CRRT circuits were used with two different haemodialysers. A high permeability polysulfone [Optiflux F-160NR, Fresenius, (Lexington, MA) surface area 1.5 m<sup>2</sup>, ultrafiltration coefficient (Kuf) 45 ml/h/mmHg, lot # 3BU341] and a high efficiency cellulose diacetate hollow fibre [CA-HP 170, Baxter (Deerfield, IL, USA) SA 1.7 m<sup>2</sup> Kuf 7.9 ml/h/mmHg, lot # E03J12]. After the extracorporeal circuit was primed, the blood was recirculated through the CRRT system for 20 min to allow for coating of the tubing and haemodialyser by blood proteins [15,16].

Dialysate was prepared by adding 240 ml of Normocarb™ sterile bicarbonate renal dialysis concentrate (Dialysis Solutions, Inc. Richmond Hills, Ontario, Canada) to a 3 l bag of sterile water (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's directions. Once reconstituted, Normocarb dialysate contains sodium 140 mEq/l, magnesium 1.5 mEq/l, chloride 106.5 mEq/l and bicarbonate 35 mEq/l. CVVHD was run in single-pass mode with a dialysate flow rate (Qd) of 33.3 ml/min. The ultrafiltrate production rate (Quf) was set at zero. Blood flow rate (Qb) was 200 ml/min (Table 1) and no adjustments in blood flow rate were necessary to maintain adequate pressures within the CRRT circuit. This process was repeated six times each with a new haemodialyser and tubing circuit for each haemodialyser membrane type.

### *In Vivo* CVVHDF

Ten adult critically ill patients receiving CVVHDF at the University of Michigan Hospital, Ann Arbor, MI, USA were enrolled from March–September 2004. The study patients were identified using the daily CRRT roster maintained in the dialysis unit of University Hospital. The study investigators obtained informed consent and complied with University of Michigan standards including patient confidentiality according to the Health Information Portability and Accountability Act (HIPAA). The study was approved by the Institutional Review Board of the University of Michigan Medical School (IRBMED) (approval # 2004–0193)

Investigators played no role in determining who received CRRT or what nutritional regimens they received. At the University of Michigan, CRRT is administered as CVVHDF with a Qd of 33.3 ml/min and sufficient ultrafiltrate production to achieve or maintain euvolaemia. Typically, adult

**Table 1.** Trace element content of study liquids

Mean ± SD	Chromium (ng/g)	Copper (ng/g)	Manganese (ng/g)	Selenium (ng/g)	Zinc (ng/g)
<i>In vitro</i> dialysate normocarb <i>n</i> = 12	0.86 ± 0.90	4.5 ± 2.2	0.95 ± 0.75	2.02 ± 1.2	38.9 ± 12.3
Tap water <i>n</i> = 1	2.4	152.9	4.5	1.4	97.4
Distilled water <i>n</i> = 1	2.7	5.4	2.1	2.1	6.8
Saline (0.9%) <i>n</i> = 1	3.0	2.6	1.3	2.7	21.7
<i>In Vivo</i> dialysate <i>n</i> = 10 patients	0.08 ± 0.17	2.0 ± 1.9	ND	2.5 ± 2.3	43.1 ± 17.2

ND: the trace element was not detectable within the limit of the assay.

patients receive CVVHDF using the same high permeability polysulfone haemodialyser (Optiflux F-160NR) used in our *in vitro* trial. Physicians could choose a smaller alternative polysulfone haemodialyser (HF1000, Gambro, Lakewood, CO, USA) with a surface area of 1.1 m<sup>2</sup> and a Kuf of 37 ml/h/mmHg.

### Sample collection

All samples in the *in vitro* and *in vivo* studies were obtained in the same manner. All samples were obtained using trace metal-free stainless steel needles (Becton Dickinson Laboratories, Franklin Lakes, NJ, USA). Blood samples were drawn at the pre-haemodialyser port (A) and the effluent/spent dialysate (EF) and dialysate (D) samples from the effluent/spent dialysate and dialysate sampling ports of the CRRT circuits, respectively. One set of samples was obtained from each study patient or *in vitro* circuit. *In vivo* samples were placed on ice immediately and transferred to the laboratory at the University of Michigan, College of Pharmacy. Each effluent and dialysate sample was split into two separate Falcon<sup>®</sup> tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) which are free of trace metals. Blood samples were transferred to Falcon<sup>®</sup> tubes and centrifuged at 3000 r.p.m for 30 min. The plasma was transferred by metal-free pipette to two Falcon<sup>®</sup> tubes and stored at -80°C until time of assay.

### Assay

For the *in vitro* study, BUN was assayed on a COBAS Integra 400 plus (Roche Diagnostics, Indianapolis, IN, USA). The lower limit of detection was 1.8 mg/dl and the coefficient of variability was < 10%. Bovine and human plasma samples (from the *in vitro* and *in vivo* experiments, respectively) were analysed for chromium, copper, manganese, selenium and zinc content at the University of Notre Dame in South Bend, Indiana using ICP-MS. The analytical technique was performed according to previously described procedures [17–19] with slight modifications using a Vacuum Generators elemental PlasmaQuad model PQ2 ICP-MS.

Frozen plasma samples (5 ml) were thawed and transferred in a pre-cleaned, pre-weighed Teflon bomb and the sample weight was recorded for each sample. Each sample was transferred from a vial to a Teflon bomb by either pouring or with the aid of a clean plastic transfer pipette. Samples were then digested in open beakers using doubly distilled concentrated nitric acid (5 ml) and hydrogen peroxide (2 ml). After the first digestion was completed a second nitric acid digestion was performed. The second digestion used beakers closed at 100°C for 12 h, to ensure complete breakdown of the organic matrix. Two ml of HNO<sub>3</sub> were added to the dried mass followed by 40 drops of H<sub>2</sub>O<sub>2</sub> in a drop-wise method to avoid foaming. The analytes were taken to 15 ml in 2% nitric acid. Nanopure 18 MΩ cm water was used for sample dilutions. Monitoring of any contaminants from reagents and laboratory environment was done with a procedural blank included with each batch.

Single element solutions (Inorganic Ventures, Lakewood, NJ, USA) were used to prepare calibration and internal standard solutions. Analyses were performed using an external calibration procedure. Scandium (Sc), cobalt (Co), gallium (Ga) and yttrium (Y) were used as internal standards for matrix and instrument drift corrections. Procedural blank

was analysed to check for any contribution from the reagents and laboratory environment. The reference materials SRM-8414 (bovine muscle) and SRM-1577b (bovine liver) were digested and analysed to be identical to the unknown for quality control purposes. The assay was able to detect each trace element at the sub-ng/g level.

### Calculations

Trace element loss was calculated by determining the extraction coefficient (EC) and transmembrane clearance of each element. Trace element and urea, *in vitro* transmembrane clearance equations for continuous haemodialysis were as follows:

$$EC = EF/A$$

$$\begin{aligned} \text{Continuous haemodialysis transmembrane} \\ \text{clearance} = EC \times Qd \end{aligned}$$

Trace element transmembrane clearance, *in vivo* involved ultrafiltrate production so the following equation was used:

Continuous haemodiafiltration

$$\text{transmembrane clearance} = EC \times (Qd + Quf)$$

A = solute concentration in plasma obtained from the pre-haemodialyser port;

EF = solute concentration in effluent/spent dialysate obtained from the effluent/spent dialysate port;

Quf = ultrafiltrate production rate;

Qd = dialysate flow rate;

Trace element loss (mg/day) = EC × mean trace element plasma concentration × total dialysate volume/day.

Trace element transmembrane clearance was calculated using the above equations. This transmembrane clearance data were used to calculate the projected daily loss of each trace element. The transmembrane clearance (ml/min) multiplied by the arterial plasma concentration (ng/g) of each trace element by 24 h gave us a calculated CRRT daily loss of each trace element. This CRRT loss for each trace element was compared with the amount supplied by a daily dose of 1 ml a common parenteral trace element supplement Multitrac<sup>TM</sup>-5 (MTE-5) Concentrate (American Regent Laboratories, Inc. Shirley, NY, USA) (Table 2). The calculated daily CRRT loss of each trace element was subtracted from the amount provided for supplementation by the MTE-5 Concentrate to determine if loss from CVVHD or CVVHDF was greater than current supplementation provided by MTE-5 Concentrate.

### Sample size and data analysis

Power analysis for the *in vitro* experiments indicated that a sample size of six haemodialysers would detect a 25% difference in trace element transmembrane clearance between haemodialyser types assuming a

**Table 2.** Trace element results of *in vitro* continuous venovenous haemodialysis (CVVHD) experiments

Haemodialyser	Chromium		Copper		Manganese		Selenium		Zinc	
	CA	F160	CA	F160	CA	F160	CA	F160	CA	F160
Extraction coefficient	0.046 ± 0.027	0.029 ± 0.008	0.006 ± 0.002	0.014 ± 0.01	0.23 ± 0.12	0.14 ± 0.12	0.023 ± 0.012	0.038 ± 0.02	0.078 ± 0.011	0.066 ± 0.01
Clearance (ml/min)	1.54 ± 0.91	0.97 ± 0.23	0.21 ± 0.07	0.47 ± 0.18*	7.8 ± 4.1	4.6 ± 3.6	0.76 ± 0.39	1.2 ± 0.63	2.7 ± 0.37	2.3 ± 0.32
Projected daily loss (mg)	0.0015	0.00089	0.0027	0.0065	0.00097	0.00056	0.0013	0.0021	0.024	0.022
Daily MTE-5 content (mg) (1 ml)	0.01		1.0		0.5		0.06		5.0	
Net daily supplementation (mg)	0.0085	0.0091	0.997	0.994	0.49	0.49	0.059	0.058	4.98	4.98
Projected percent of daily dose removed by CVVHD	15%	9%	0.3%	0.6%	2%	2%	1.7%	3.3%	0.4%	0.4%

CA: Baxter CA-HP 170 haemodialyser; F160: Fresenius Optiflux 160 haemodialyser; Clearance: transmembrane clearance;

Net daily supplementation: (trace element amount in a typical daily dose) – (projected daily CRRT loss).

\* *P* value < 0.05 comparing CA and F-160 haemodialyser using a *t*-Test 2 tailed with unequal variance.

10% standard deviation with a power of 0.9 and a significance level of  $P < 0.05$ . The mean EC (with corresponding transmembrane clearance values) between the two types of haemodialysers for each trace element were compared using ANOVA. Power analysis for the *in vivo* portion of the study was not conducted, but sample size was consistent with previous CRRT trace element clearance studies [7–9].

## Results

Trace element content of the liquids (*in vivo* dialysate, normal saline, distilled water, Normocarb *in vitro* dialysate and tap water as a control) used in the *in vitro* experiment was assessed to determine a possible source of additional trace elements that could confound our findings (Table 1). The custom dialysate used in patients is made by a commercial vendor for the hospital. The *in vivo* dialysate had the highest concentration of zinc (43.1 ng/g) with much lower concentrations of the other elements present. The Normocarb dialysate used in the *in vitro* experiments had relatively higher concentrations of zinc (38.9 ng/g) than the other trace elements. Overall the trace element profiles were similar between the *in vitro* Normocarb dialysate and *in vivo* hospital dialysate (Table 1).

### *In vitro* CVVHD model

All experiments were conducted as outlined in the methods and there were no circuit failures during the *in vitro* continuous haemodialysis experiments. The mean ± SD urea EC for the cellulose diacetate haemodialyser was  $0.87 \pm 0.21$  and for the polysulfone haemodialyser  $0.98 \pm 0.07$ . Table 2 contains the EC data for each trace element. The mean *in vitro* EC from the cellulose diacetate haemodialyser tended to be higher for chromium, manganese and zinc than with the polysulfone haemodialyser but these differences were not statistically significant. The polysulfone haemodialyser had higher EC for copper and selenium but only copper's EC difference was statistically significant from the cellulose diacetate haemodialyser ( $P < 0.02$ ).

Based on the mean EC derived from these *in vitro* data, we predicted trace element transmembrane clearance for each haemodialyser using the experimental conditions (dialysate flow rate of 33.3 ml/min and no ultrafiltrate production). The amount of predicted daily trace element CRRT loss, calculated from the *in vitro* mean plasma trace element blood concentration and *in vitro* CRRT transmembrane clearance values appears in Table 2. The amount of daily trace element supplementation provided by a common, commercially available trace element product (MTE-5, components shown in Table 2) was subtracted from the calculated daily trace element loss to determine if CVVHD at 33.3 ml/min would result in

**Table 3.** CVVHDF patient demographics

Patient	Sex	Age (years)	Weight (kg)	Cause of acute renal failure	Reason for CRRT	Days on CRRT prior to sample	Time on current haemodialyser prior to sample	Nutrition
1	F	59	140	Sepsis	ARF	11 days	14 h 30 min	Enteral
2	F	57	64	ESRD	Fluid overload	12 days	18 h	Enteral
3	M	57	77	Hepato-renal	Fluid overload	13 days	10 h 50 min	PN
4	M	35	37	ESRD	Hypotensive	11 days	16 h	Enteral
5	F	18	65	Sepsis	Acidosis	4 days	35 h	PN
6	M	34	110	Cardio-renal	Fluid overload	14 h 20 min	14 h 20 min	Enteral
7	M	70	90	Heart failure	ARF	43 h	43 h	none
8	M	52	84	ARF	ARF	25 h	25 h	Enteral
9	M	41	NR	Sepsis	ARF	6 months	27 h	Enteral
10	F	54	55	Sepsis	ARF	4 days	30 h	Enteral

ARF: Acute renal failure; ESRD: End-stage renal disease; PN: Parenteral nutrition.

**Table 4.** Patient trace element (TE) plasma concentrations compared with *in vitro* experiment bovine plasma values

TE Arterial plasma concentrations	Chromium ng/g	Copper ng/g	Manganese ng/g	Selenium ng/g	Zinc ng/g
Patient 1	37.9	693.7	1.9	99.2	563.9
Patient 2	31.7	1241.3	1.6	92.9	597.8
Patient 3	27.8	923.9	1.5	86.2	675.9
Patient 4	34.1	874.4	1.5	60.4	497.9
Patient 5	29.5	952.6	7.3	170.6	1344.8
Patient 6	32.9	2362.9	1.6	111.8	681.8
Patient 7	32.9	1701.9	18.7	109.8	1028.7
Patient 8	22.0	1085.7	0.6	88.5	586.4
Patient 9	35.1	133.7	0.7	50.4	371.3
Patient 10	36.0	337.3	3.3	92.9	535.1
Mean $\pm$ SD	32.0 $\pm$ 4.6	1030.7 $\pm$ 642.4	3.9 $\pm$ 5.5	96.3 $\pm$ 32.5	688.4 $\pm$ 287.0
Range	22.0–37.9	133.7–1701.7	0.6–18.7	50.4–170.6	371.3–1344.8
Bovine plasma Mean $\pm$ SD	38.1 $\pm$ 1.04	575.7 $\pm$ 19.8	5.1 $\pm$ 0.40	68.4 $\pm$ 3.7	399.6 $\pm$ 14.8

**Table 5.** *In vivo* experiments: trace element continuous venovenous haemodiafiltration (CVVHDF) calculations

	Chromium	Copper	Manganese	Selenium	Zinc
Extraction coefficient	0.13 $\pm$ 0.06	0.01 $\pm$ 0.007	0.05 $\pm$ 0.1	0.04 $\pm$ 0.03	0.1 $\pm$ 0.03
Clearance (ml/min)	5.4 $\pm$ 2.4	0.45 $\pm$ 0.33	1.9 $\pm$ 4.6	1.6 $\pm$ 1.2	4.0 $\pm$ 1.3
Daily loss (mg)	0.0042	0.011	0.0002	0.0037	0.069
Daily MTE-5 supplement (mg) (1 ml)	0.01	1.0	0.5	0.06	5.0
Net daily supplementation (mg)	0.0058	0.99	0.499	0.056	4.93
Projected percent of daily dose removed by CVVHDF	42%	1%	0.2%	6.7%	1.4%

Clearance: transmembrane clearance; Net daily supplementation: (trace element amount in a typical daily dose)–(projected daily CRRT loss).

a net daily loss of any trace element even if conventional trace element supplementation is given.

#### *In vivo* CVVHDF study

Adults who received CVVHDF were a diverse group. Large differences in duration of CVVHDF, patient weight, age and type of nutrition received were apparent (Table 3). The mean  $\pm$  SD Q<sub>b</sub> was 173  $\pm$  25 ml/min. The mean  $\pm$  SD Q<sub>d</sub> was 34.2  $\pm$  2.7 ml/min. The mean ultrafiltrate production rate in these patients was 7.65  $\pm$  3.05 ml/min to achieve

or maintain euvolaemia. All patients received anti-coagulation with sodium citrate according to the University of Michigan protocol [20]. Nine patients received CVVHDF using the Optiflux F160 NR hemodialyser and one patient (patient 5) received treatment with a Prisma HF1000 hemodialyser.

The arterial plasma concentrations of the five trace elements varied widely in our patients (Table 4). Copper and zinc showed the most inter-patient variability. The *in vivo* EC and trace element CRRT transmembrane clearance data appear in Table 5. Daily CRRT loss calculations indicate that very little copper, manganese, selenium and zinc were removed

by CVVHDF. Indeed, manganese was not detectable in the effluent of 8/10 patients. Selenium was not detectable in the effluent of 2/10 patients, consequently the calculated CVVHDF transmembrane clearance of manganese and selenium was considered to be zero in these patients. Substantially more chromium was removed than other trace elements; however the daily CVVHDF loss was less than half of what would be supplemented with a daily dose of MTE-5 (Table 5).

## Discussion

We were able to quantify the transmembrane clearance of five trace elements in critically ill patients receiving CVVHDF. The emergence of ICP-MS assay technology has allowed for the measurement of trace elements to the sub-ng/g level. Most of the previous studies of trace element disposition in patients receiving CRRT were conducted with less sensitive assays [21,22]. A later study by Berger used ICP-MS, but these authors did not report manganese and chromium values [23]. These more sensitive assays allow us to better characterize trace element disposition in CRRT.

Our study patients came from many different intensive care units in our hospital and consequently were a heterogeneous population. Plasma serum concentrations of trace elements likely differ between different types of critically ill patients and otherwise healthy individuals. Our one-time sampling strategy provides a snapshot of the trace element transmembrane clearance in these ten patients, many of whom were receiving CVVHDF for more than a week. Our methodology used a single plasma sample, which, for the purposes of calculating daily losses, we assumed was representative of the plasma concentration for an entire day. However, it is likely that critically ill patients have variability in their trace element plasma concentrations depending on underlying illness and state of inflammation or infection.

The differences in patient characteristics may partially explain the wide variety in observed trace element profiles (Table 4). For instance, study patient 9 had the lowest plasma copper, selenium and zinc concentrations. This individual received CRRT for 6 months to treat ARF resulting from trauma and severe burns from a motor vehicle accident. This prolonged hospitalization likely influenced his trace element profile. Other study patients with disparate trace element concentrations include study patient 8, who was post-liver transplant from hepatitis and had received one day of CRRT at the time of sampling. This enterally fed individual had the lowest manganese and chromium plasma concentrations. Study patient 6, a 43-year-old male with congestive heart failure and cardiomyopathy awaiting a heart transplant, had the highest copper value at twice the population mean. This patient also had the shortest duration (14 h) of CVVHDF at the time of sampling. Lastly, study patient 5 was an 18-year-old female with a recent bone marrow transplant and acute renal failure attributed

to sepsis. This patient had an elevated serum zinc concentration at approximately twice the population mean and was receiving daily trace element supplementation with MTE-5.

Comparison of *in vitro* and patient-derived trace element transmembrane clearance values in the present study indicates agreement between *in vitro* polysulfone and *in vivo* polysulfone EC and transmembrane clearance (Tables 2 and 5). The trace elements chromium and zinc exhibited a statistically significant difference in transmembrane clearance between *in vitro* and *in vivo* investigations ( $P < 0.01$ ). The larger *in vivo* transmembrane clearances might be partially explained by the fact that the *in vitro* study utilized only 33.3 ml/min of dialysate flow, while the CVVHDF patients received 33.3 ml/min of dialysate flow plus an additional  $7.7 \pm 3.1$  ml/min of ultrafiltrate production providing additional convective transmembrane clearance. Although statistically significant, the amount of chromium and zinc removed by CVVHDF was substantially below what is typically supplemented. These differences may also be explained by variations in protein binding and tissue distribution in study patients compared with the *in vitro* setting. *In vitro* experiments were run with blood obtained from healthy cows. Bovine blood could differ from critically ill human blood in terms of trace element concentrations and to the extent that these trace elements bind to plasma proteins (and consequently, how available they are to be removed by CRRT). Inflammation can influence the expression of trace element containing proteins in the plasma as well as free trace element concentrations. Consequently, the fact that our patients were critically ill but the bovine blood came from otherwise healthy cattle, may account for some differences between the *in vivo* and *in vitro* studies. Table 4 lists the mean bovine plasma trace element concentration values and the plasma trace element values from each of the patients in the *in vivo* part of the study. We observed a wide range of trace element concentrations in critically ill patients. Nonetheless, there are substantial trace element differences between the critically ill human and the bovine plasma obtained from otherwise healthy cattle. The extent of trace element protein binding can be inferred from EC values [24]. EC is an indirect measurement of plasma protein binding. The *in vitro* polysulfone experiment EC values were compared with the EC values obtained in patients who also were treated with polysulfone haemodialysers. The EC values for chromium and zinc differed significantly between bovine and human values. Calculations from our bovine blood-based *in vitro* study suggested that only small amounts of trace elements would be removed by CVVHD and our *in vivo* study corroborated those projections. However, there were large differences between trace element plasma concentrations and EC values limiting the predictive value of the *in vitro* bovine blood-based model.

The *in vitro* portion of the study allowed us to compare the performance of two common CRRT

haemodialysers F-160NR (polysulfone) and the CA-HP 170 (diacetate). The surface area between these two haemodialysers is similar but the ultrafiltration coefficient differences indicate the polysulfone haemodialyser is more permeable. For small solutes like urea, there are unlikely to be differences in EC. Indeed, the urea EC approached one for both haemodialyser types and is consistent with previously published data [21,22]. CRRT transmembrane clearance differences between haemodialysers are commonly observed in most drug studies [21,23], as most drugs have larger molecular weights than urea. However, the present study did not find many trace element EC differences between haemodialyser types. This is likely due to the fact that trace elements are highly protein bound and were minimally removed by either haemodialyser type. Typically, if large transmembrane clearance differences are observed between CRRT haemodialyser types, they are observed with larger molecular weight substances [14,21].

Our *in vitro* calculations were substantiated by the *in vivo* study, namely that supplementation with standard doses of MTE-5 results in more than enough trace elements to overcome the amount lost by CVVHD. However, there were limitations to this study. For the *in vivo* investigation of this study, we did not quantify the loss of trace elements in the faeces, urine or wounds of our patients. Table 3 indicates that nine of the 10 study patients were receiving nutrition, but not all study patients were receiving the same standardized nutrition. Two patients received parenteral nutrition and the remaining seven patients were fed a variety of enteral nutrition formulations during CRRT therapies. Another potential limitation is that our *in vitro* study employed CVVHD while our study subjects received CVVHDF. This additional convective clearance could influence EC. Nonetheless, the agreement between the reported patient derived and *in vitro* transmembrane clearance demonstrate that this *in vitro* bovine blood model can be used to generate initial CRRT transmembrane clearance estimates.

While we did not directly measure non-CRRT trace element losses, it appears that MTE-5 standard doses are sufficient to replace CVVHDF losses. However, whether the MTE-5 daily dose is the optimal dose has been recently called into question. Angstwurm *et al.* [25] recently reported that higher doses of selenium resulted in improved mortality rates in critically ill patients with septic shock.

## Conclusion

Measuring five trace elements with a very sensitive ICP-MS assay, our *in vitro*, bovine blood-based CVVHD model demonstrated that these trace elements could be cleared by CVVHD with high or low permeability haemodialysers. Despite this transmembrane clearance, we calculate that standard trace element supplementation such as MTE-5 that is used in parenteral nutrition would more than replace

the trace element CVVHD losses. Our follow-up clinical study conducted in 10 critically ill patients receiving CVVHDF validated this finding. Daily trace element supplementation exceeds trace element losses attributed to CVVHDF with a dialysate flow rate of 33.3 ml/min.

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**Conflict of interest statement.** None declared.

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