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CLINICAL STUDY

# Is Circulating Phospholipase $A_2$ Removed by Large-Pore Continuous Venovenous Hemodiafiltration in Septic Acute Renal Failure?

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## ABSTRACT

Group II A phospholipase $A_2$ ($PLA_2$) produces many inflammatory lipid mediators, and the elevation in the level during sepsis has been correlated positively with the decrease in the arterial blood pressure. We studied the effect of large-pore continuous venovenous hemodiafiltration (LP-CVVHDF) on the plasma $PLA_2$ concentration and the clearance mechanism during septic acute renal failure. The subjects were 10 consecutive patients with septic acute renal failure receiving CVVHDF. Simultaneous samples of arterial, and filter inlet and outlet blood, and ultradiafiltrate were collected before starting CVVHDF (0 hr), and 4 hr, 12 hr and 24 hr after starting CVVHDF. $PLA_2$ activity was measured in plasma and ultradiafiltrate. We eluted $PLA_2$ bound to hemofilter from patient and the classification of $PLA_2$ type of eluting solution and ultradiafiltrate was done using Western blot analysis. Plasma clearance (mL/min) was $28.1 \pm 7.6$ at 4 hr, $23.2 \pm 8.9$ at 12hr and $17.5 \pm 8.0$ at 24 hr. Plasma clearance at 4 hr was higher than that at either 12 hr or 24 hr. Plasma clearance mainly consisted of adsorption by LP-CVVHDF. The changes in arterial plasma $PLA_2$ activity were not statistically significant. One mg/mL of heparin eluted $PLA_2$ bound to the large-pore hemofilter. The $PLA_2$ in eluting solution and in ultradiafiltrate were identified as an approximately 70 kD band in Western blot analysis using anti-human

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secretory II A-$PLA_2$ monoclonal antibody. The results show that circulating $PLA_2$ can be removed by adsorption with LP-CVVHDF to some extent and that plasma $PLA_2$ activity is not significantly decreased. Because $PLA_2$ clearance with LP-CVVHDF is estimated as <1% of total body $PLA_2$ clearance, LP-CVVHDF could not be a clinically efficient therapy to remove the circulating $PLA_2$.

*Key Words:* Continuous hemodiafiltration; Sepsis; Acute renal failure; Adsorption; Phospholipase $A_2$; Clearance; Large-pore membrane; Western blot analysis.

## INTRODUCTION

Group II A phospholipase $A_2$ ($PLA_2$), a 14kD enzyme, is a key enzyme in the production of lipid mediators (prostaglandin $I_2$, thromboxane $A_2$, leukotriene $B_4$, platelet-activating factor, etc.) from membrane phospholipids when the inflammatory cascade is activated.[1] $PLA_2$ activity was reported to be elevated 35-fold in the plasma of septic patients, and the measurement of plasma $PLA_2$ is recommended to estimate the individual risk for occurrence of lethal multiple organ failure.[2] The elevation in the level of plasma $PLA_2$ during sepsis has been reported to correlate positively with the decrease in the mean arterial blood pressure.[3] Thus, the removal of circulating $PLA_2$ might have a therapeutic significance in septic patients.

Continuous venovenous hemodiafiltration (CVVHDF) is well suited to the unstable hemodynamic conditions of septic patients complicated with acute renal failure. Although the removal of various inflammatory mediators by CVVHDF became very attractive therapy, it is controversial whether CVVHDF could remove these mediators.[4] The removal of cytokines by CVVHDF is mainly due to hemofilter membrane adsorption, and the adsorption is estimated according to calculation from differences between prefilter and postfilter concentrations of cytokines.[5]

Large-pore CVVHDF (LP-CVVHDF) has a larger pore size than the previous standard membrane (cutoff point: 30–50 kD) to permit efficient elimination of various soluble mediators with 10 kD–50 kD molecular sizes, and was reported to improve cardiovascular function during endotoxin shock.[6] However, it is unknown whether LP-CVVHDF could remove circulating $PLA_2$ clinically effectively.

The present study was carried out to determine if $PLA_2$ could be removed by LP-CVVHDF clinically effectively, to clarify the clearance mechanisms and to prove directly that the hemofilter membrane adsorbs circulating $PLA_2$ in patients with septic acute renal failure.

## MATERIALS AND METHODS

### Patients

This study was approved by the Ethics Committee of the Nagasaki University School of Medicine and conducted in the intensive care unit (ICU) of Nagasaki University Hospital from July 1999 to July 2000. Informed written consent was obtained from each patient or the relatives. The subjects were 10 consecutive patients with septic acute renal failure receiving LP-CVVHDF. Sepsis was diagnosed according to the criteria of American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.[7] The diagnosis of acute renal failure was based on the decreased renal function in need of dialysis,[8] as defined by a rise of the serum creatinine >3 mg/100 mL and a urine output <20 mL/hr despite volume correction and intensive diuretic therapy. The severity of the disease was assessed using APACHE II score.[9] The Glasgow Coma Scale was excluded from the calculation of the APACHE II score, as some patients were sedated and intubated.

### Therapy

All patients received conventional intensive care therapy according to clinical requirements. Vasopressor agents (dopamine, dobutamine, norepinephrine, etc.) were adjusted by the attending physician to maintain an adequate mean arterial blood pressure. Antibiotics indicated by microbiological tests were administered intravenously. All intubated patients were sedated with a continuous infusion of buprenorphine and midazolam. The ventilator setting was adjusted by the attending physician to maintain clinically appropriate gas exchange.

### CVVHDF

The femoral vein was cannulated for vascular access with an 11-Fr, double-lumen catheter. CVVHDF was performed using a large pore polyacrylonitrile

(PAN) hemofilter (APF-06S, Asahimedical, Tokyo, Japan). This membrane has the cut-off point of approximately 55–65 kD, the mean pore size of 8.3 nm and the effective surface area of 0.6 $m^2$. The study was carried out during the first 24 hr of treatment with the single hemofilter. Blood was pumped through the hemofilter at 80 mL/min ($Q_B$) by a CVVHDF peristaltic pump system, and was then returned to the circulation. We used a bicarbonate dialysate. The bicarbonate dialysate was simultaneously infused through the hemofilter, countercurrent to blood flow, at a constant rate of 500 mL/hr ($Q_D$). The ultrafiltration rate was 500 mL/hr ($Q_F$) and the same bicarbonate replacement solution was administered at a rate of 400–500 mL/hr ($Q_R$) in a postfilter fashion. The extracorporeal circuit was protected against coagulation with nafamostat mesylate (Futhan, Toriiyakuhin, Tokyo, Japan) or heparin in the afferent limb. The infusion of anticoagulants was adjusted to maintain the activated clotting time (ACT) about 150 sec. Simultaneous samples of arterial, and filter inlet and outlet blood, and ultradiafiltrate were collected before starting CVVHDF (0 hr), and 4 hr, 12 hr and 24 hr after starting CVVHDF. At each point, hemodynamic determinations, arterial blood gas analysis and hematological analysis were performed.

## Control

We collected arterial samples from 7 control patients (age 56–73, 4 females and 3 males) who underwent total knee replacement surgery without collagen disease. After general anesthesia and cannulation into radial artery, we drew samples for $PLA_2$ assay.

## $PLA_2$ Analysis

Samples were centrifuged at 3000 rpm for 10 min, plasma was diluted 50-fold in buffer consisting of 50 mM Tris, 150 mM NaCl, 1 mM EDTA and 1 mM EGTA, that contained the protease inhibitors, 20 μM leupeptin and 0.1 mM phenylethyl sulfonyl fluoride (PMSF). Diluted plasma and ultradiafiltrate were stored at −80°C until assayed.

$PLA_2$ activity was measured as the previously described manner with some modification.[10] L-3-phosphatidylethanolamine, 1-acyl-2- [1−$^{14}$C] arachidonyl (PE) (Amersham, Buckinghamshire, UK) was used as exogenous substrate, which was dried under $N_2$ and resuspended in ethanol. The $PLA_2$ assay buffer (100 μL) contained 75 mM Tris-HCl, 10 mM $CaCl_2$, and 0.22 nmol of the PE (∼25,000 cpm) at pH 9.0. The reaction was carried out at 37°C for 30 min and was stopped by adding 0.56 mL of Dole's reagent: 48.75% isopropyl alcohol, 50% n-heptane, 1.25% 1N $H_2SO_4$ in water. Arachidonic acid (AA) was extracted in the following manner. Water, 0.11 mL, was added and the sample was vortexed and centrifuged at 10,000g for 5 min. A volume of 0.15 mL of the upper phase was transferred to a new tube to which 50 μL silica gel and 0.8 mL of n-heptane were added. The samples were vortexed and centrifuged again for 5 min each. A volume of 0.8 mL of supernatant was then counted in a liquid scintillation counter. $PLA_2$ activity was expressed as pmol of radiolabeled AA released from PE per min per mL of plasma.

## Calculations

The following formulas were used:

Plasma flow (mL/min)

$$Q_{Pin} = Q_B \times (1 - Hct_{in}/100)$$
$$Q_{Pout} = [QB - (Q_F - Q_R)/60] \times (1 - Hct_{out}/100)$$
$$\text{Sieving Coefficient} = C_{uf}/C_{in}$$

Clearance (mL/min)

Plasma $PLA_2$ clearance
$$(PCr) = [(Q_{Pin} \times C_{in}) - (Q_{Pout} \times C_{out})]/C_{in}$$
Ultradiafiltrate $PLA_2$ clearance
$$(UCr) = (Q_F + Q_D)/60 \times C_{uf}/C_{in}$$
Adsorption $PLA_2$ clearance
$$(ACr) = PCr - UCr$$

where $Q_B$ is blood flow rate (mL/min); $Q_F$ is ultrafiltrate flow rate (mL/hr); $Q_D$ is dialysate flow rate (mL/hr); $Q_R$ is replacement fluid flow rate (mL/hr); $Q_{Pin}$ is prefilter plasma flow rate (mL/min); $Q_{Pout}$ is postfilter plasma flow rate; $Hct_{in}$ is hematocrit (Hct) in inlet blood; $Hct_{out}$ is Hct in outlet blood; $C_{uf}$ is $PLA_2$ activity in ultradiafiltrate (pmol/min/mL); $C_{in}$ is $PLA_2$ activity in inlet plasma; and $C_{out}$ is $PLA_2$ activity in outlet plasma.

## Elution of $PLA_2$ Bound to Large-Pore Hemofilter

One LP-CVVHDF hemofilter was removed from No.9 patient 4 hr after starting CVVHDF the next day after time-course experiment. One thousand mL of

*Table 1.* Patient characteristics.

| Patient | Gender | Age (years) | BW (kg) | AP- II score | Pathology | Outcome |
|---|---|---|---|---|---|---|
| 1 | Male | 74 | 63 | 18 | CABG, aortic-abdominal graft | Died |
| 2 | Female | 58 | 34 | 16 | Bronchoesophageal fistula | Died |
| 3 | Female | 73 | 57 | 10 | MNMS | Survived |
| 4 | Male | 86 | 56 | 17 | Burn | Died |
| 5 | Male | 57 | 60 | 17 | Crush syndrome | Died |
| 6 | Female | 60 | 64 | 11 | CABG | Survived |
| 7 | Male | 80 | 42 | 14 | CABG | Died |
| 8 | Male | 73 | 39 | 15 | Mitral valve replacement | Died |
| 9 | Male | 76 | 45 | 20 | Malignant lymphoma | Survived |
| 10 | Female | 71 | 47 | 20 | Pulmonary embolism | Survived |

BW, body weight; AP- II, acute physiology and chronic health evaluation II; CABG, coronary artery bypass graft; MNMS, myonephropathic metabolic syndrome.

acetate Ringer solution was pumped through the hemofilter at 100 mL/min without convection or dialysis, to wash the hemofilter. Ten 5-mL fractions every 100 mL of washed solution (fraction number: F1 to F10) were collected. And then, two hundred mL of saline including 1 mg/mL heparin[11] was pumped through the hemofilter at 100 mL/min to elute $PLA_2$ from the hemofilter. Two 5-mL fractions every 100 mL of eluting solution (fraction number: H1, H2) were collected. The protease inhibitors, 20 μM leupeptin and 0.1 mM PMSF, were added to each fraction and fractions were centrifuged at 3000 rpm for 10 min. Protein concentration of each fraction was measured using a protein analysis kit (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as a standard and $PLA_2$ activity of each fraction was assayed.

## Western Blot Analysis

Two mL of ultradiafiltrate from No. 9 patient was concentrated to 200 μL using Centricon 10 (Amicon, Danvers, MA). Proteins in F10, H1 and concentrated ultradiafiltrate were denatured and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (15% polyacrylamide gradient gel).[12] Human synovial $sPLA_2$ (purified group II A $PLA_2$) (Cayman Chemical, Ann Arbor, MI) was used as a standard. After SDS-PAGE, separated proteins were electrophoretically transferred to a polyvinylidene fluoride (PVDF) (PVDF-PLUS, Micron Separations, Westborough, MA) membrane in 25 mM Tris-HCl (pH=8.3), 190 mM glycine and 20% methanol. Nonspecific binding of anti-human group II A $PLA_2$ antibody to PVDF membrane was prevented by preincubation of the membrane in 5% skim milk in Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 8.0, 137 mM NaCl) for an hr at room temperature. The blocked PVDF membrane was incubated with a 1: 200 dilution of a mouse anti-human group II A $PLA_2$ antibody (Upstate Biotechnology, Lake Placid, NY) for 2 hr at room temperature with constant shaking. Unbound antibodies were removed with three washes of TBST

*Table 2.* Parameters of phospholipase $A_2$ clearance (mean±SD) of LP-CVVHDF.

| | Time after LP-CVVHDF (hr) | | | |
|---|---|---|---|---|
| Parameters | 0 | 4 | 12 | 24 |
| Plasma $PLA_2$ activity | 53.9±40.1 | 46.5±33.6 | 55.0±40.7 | 61.2±51.8 |
| Sieving coefficient | | 0.03±0.03 | 0.05±0.05 | 0.08±0.13 |
| Plasma $PLA_2$ CL | | 28.1±7.6 | 23.2±8.9[a] | 17.5±8.0[a] |
| Ultradiafiltrate $PLA_2$ CL | | 0.4±0.4 | 0.9±0.9 | 1.4±2.2 |
| Adsorption $PLA_2$ CL | | 27.7±7.9 | 22.3±8.8[a] | 16.0±8.3[a] |

$PLA_2$, phospholipase $A_2$ (pmol/min/mL); CL, clearance (mL/min); LP-CVVHDF, large-pore continuous venovenous hemodiafiltration.
[a]$p<.05$ compared with parameter at 4 hr.

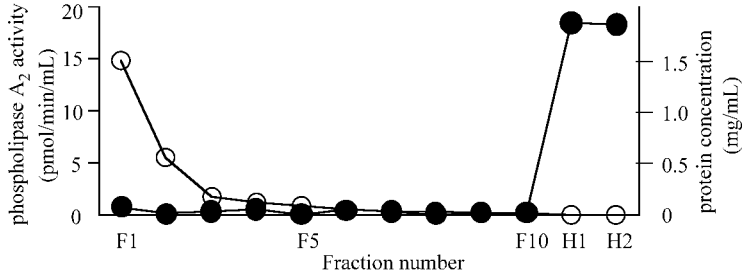


***Figure 1.*** Phospholipase $A_2$ ($PLA_2$) activity and protein concentration after heparin elution from large-pore hemofilter. One hemofilter was washed with 1 L of acetate Ringer solution and ten 5-mL fractions every 100 mL of washed solution (fraction number: F1 to F10) were collected. $PLA_2$ bound to hemofilter was eluted with 200 mL of saline including 1 mg/mL heparin and two 5-mL fractions every 100 mL of eluting solution (fraction number: H1, H2) were collected. Protein concentration (open circle) and $PLA_2$ activity (solid circle) of each fraction were assayed.

(TBS containing 0.5% Tween 20). The PVDF membrane was incubated with a 1:3000 dilution of a rabbit anti-mouse immunoglobulin, peroxidase-linked species-specific whole antibody (Wako, Osaka, Japan) for 1 hr at room temperature. The sites of antibody binding were developed with an enhanced chemiluminescence system (LuminGLO, New England Biolabs, Beverly, MA).

### Statistical Analysis

All data of $PLA_2$ activity were obtained as the average of triplicate measurements. Results were presented as mean±SD. Results were evaluated by Kruskal-Wallis and Wilcoxon test with $P<0.05$ regarded as significant.

## RESULTS

### Patients

The characteristics of the patients are summarized in Table 1. The mean APACHE II score, mean age (years) and mean body weight (kg) were 16.5±3.9, 70.8±9.6 and 50.7±10.6, respectively. Four patients (40%) survived to leave the ICU. Six patients (60%) died from irreversible multiple organ distress syndrome (MODS) following sepsis.

### Control

In the control group, the plasma $PLA_2$ activity was 2.5±1.9 pmol/min/mL.

### Hemofilter Data

The kinetic property of $PLA_2$ under LP-CVVHDF is shown in Table 2. There were no significant differences in the plasma $PLA_2$ activity among the time point. There were no significant changes in $PLA_2$ sieving coefficient of LP-CVVHDF during the time course. Plasma $PLA_2$ clearance (mL/min) by LP-CVVHDF was 28.1±7.6 at 4 hr, 23.2±8.9 at 12 hr and 17.5±8.0 at 24 hr. Plasma $PLA_2$ clearance at 4 hr was higher than that at either 12 hr or 24 hr. Ultradiafiltrate $PLA_2$ clearance by LP-CVVHDF did not change throughout the time course. Adsorption $PLA_2$

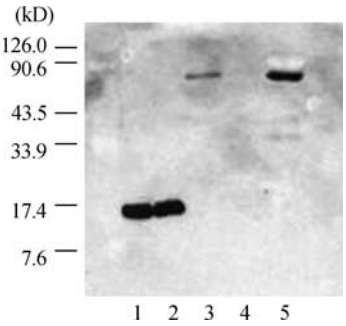


***Figure 2.*** Western blot analysis of the eluted phospholipase $A_2$ ($PLA_2$) using anti-human group IIA $PLA_2$ monoclonal antibody. The purified IIA $PLA_2$ (lane 1) and purified $PLA_2$ including 1 mg/mL heparin (lane 2) were identified as one approximately 14 kD band, and the $PLA_2$ in eluting solution (H-1) (lane 3) was identified as an approximately 70 kD band. There was no band in washing solution (F-10) (lane 4). The $PLA_2$ in the ultradiafiltrate was identified as an approximately 70 kD band (lane 5).

clearance (mL/min) at 4 hr (27.7±7.9) was higher than that at either 12 hr (22.3±8.8) or 24 hr (16.0±8.3).

### Elution of $PLA_2$ Bound to Large-Pore Hemofilter

As shown in Fig. 1, the washing solution and eluting solution diluted plasma protein concentration in each fraction, gradually. Although $PLA_2$ activities in the washed solution fractions except F1 were close to 0, $PLA_2$ activities in the eluted solution fractions elevated to above 15 pmol/min/mL. Thus, 1 mg/mL of heparin in eluting solution would have eluted $PLA_2$ bound to large pore hemofilter.

### Western Blot Analysis

In order to confirm that eluted $PLA_2$ from the hemofilter was group II A $PLA_2$, the classification of $PLA_2$ type was done using Western blot analysis with anti-human group II A $PLA_2$ monoclonal antibody. As shown in Fig. 2, the purified II A $PLA_2$ and purified $PLA_2$ including 1mg/mL heparin were identified as an approximately 14 kD band in Western blot analysis, and the $PLA_2$ in eluting solution (H-1) was identified as an approximately 70 kD band. There was no band in F-10. The $PLA_2$ in the ultradiafiltrate was identified as an approximately 70 kD.

## DISCUSSION

The present results show that plasma $PLA_2$ activity can be removed by adsorption with LP-CVVHDF to some extent and the efficiency of adsorption depends on the time, and that plasma $PLA_2$ activity is not significantly decreased. Because $PLA_2$ sieving coefficient of LP-hemofilter is less than 0.1, the hemofilter cannot convey circulating $PLA_2$.

Although there was no previous report concerning kinetic properties of group II A $PLA_2$ in humans, kinetic properties of the enzyme in rat were reported previously.[13] Murakami et al. injected purified group II A $PLA_2$ labeled with $^{125}I$ into the rat intravenously, and measured the half-life of the enzyme. They demonstrated that the radioactivity disappeared rapidly from the blood stream, and that it remained 17.4% of the injected radioactivity 1 min and 0.4% 10 min after injection. Assuming that the body weight of rat, total blood volume and hematocrit are 0.45kg, 7% and 47%, respectively, total body $PLA_2$ clearance is calculated using one-compartment model. Total body $PLA_2$ clearance in the rat was 26.5 mL/min. Adapting the clearance in the rat to human, total body $PLA_2$ clearance in human corrected by body weight is about 3000 mL/min. $PLA_2$ clearance with LP-CVVHDF is estimated as<1% of total body $PLA_2$ clearance in human. With regard to drug removal, it is generally accepted that extracorporeal clearance is clinically significant if its contribution to total body clearance exceeds 25–30%.[4]

Yekebas et al. induced pancreatitis by a combined intraductal injection of sodium taurocholate and enterokinase in pig.[14] They compared the pigs treated with continuous venovenous hemofiltration (CVVHF) with those without CVVHF. CVVHF significantly prolonged survival time and improved hemodynamic parameters and gas exchange. CVVHF significantly attenuated an increase in $PLA_2$ concentrations induced by pancreatitis. The sieving coefficient of their membrane was 0.7–0.8. Estimating from the sieving coefficient of myoglobin (17.8kD)[15] which is a larger molecular weight than II A $PLA_2$ (14 kD), the expected II A $PLA_2$ sieving coefficient of LP-CVVHDF is greater than 0.85. However, the present $PLA_2$ sieving coefficient of LP-hemofilter is less than 0.1. Our present result shows that purified group II A $PLA_2$ has a molecular weight of 14 kD. However, the circulating $PLA_2$ filtered to the ultradiafiltrate and bound to the membrane whose molecular weight are approximately 70 kD precludes important passage through the LP-hemofilter.

It has been reported that the activity of group II A $PLA_2$ increases in the serum of septic patients. Murakami et al. demonstrated that group II A $PLA_2$ had capacities for binding to cell surface and heparin Sepharose chromatography, and that high concentrations of either salt (1M) or heparin (1 mg/mL) solubilized membrane associated group II A $PLA_2$.[11] We solubilized circulating $PLA_2$ bound to hemofilter and Western blot analysis shows that solubilized $PLA_2$ is identified as an approximately 70 kD band. $PLA_2$ in the ultradiafiltrate is identified as an approximately 70 kD band. This monoclonal antibody cannot cross-react with other mammalian $PLA_2$, and Western blot analysis shows that purified $PLA_2$ including 1 mg/mL heparin were identified as one approximately 14 kD band. Circulating group II A $PLA_2$ might be bound to some substance or be an oligomer, according to the present Western blot results.

In conclusion, circulating group II A $PLA_2$ can be removed by adsorption with LP-CVVHDF to some extent and the efficiency of adsorption depends on the

time. However, LP-CVVHDF cannot affect plasma $PLA_2$ activity, and thus LP-CVVHDF is not a clinically efficient therapy to remove the circulating $PLA_2$.

## REFERENCES


1. Bulger, E.M.; Maier, R.V. Lipid mediators in the pathophysiology of critical illness. Crit. Care Med. **2000**, *28* (4), N27–N36. Suppl.
2. Uhl, W.; Beger, H.G.; Hoffmann, G.; Hanisch, E.; Schild, A.; Waydhas, Ch.; Entholzner, E.; Müller, K.; Kellermann, W.; Vogeser, M.; Leskopf, W.; Zügel, M.; Busch, E.W.; Büchler, M.W. A multicenter study of phospholipase $A_2$ in patients in intensive care unit. J. Am. Coll. Surg. **1995**, *180* (3), 323–331.
3. Vadas, P.; Pruzanski, W.; Stefanski, E.; Sternby, B.; Mustard, R.; Bohnen, J.; Fraser, I.; Farewell, V.; Bombardier, C. Pathogenesis of hypotension in septic shock: correlation of circulating phospholipase $A_2$ levels with circulatory collapse. Crit. Care Med. **1988**, *16* (4), 1–7.
4. Schetz, M.; Ferdinande, P.; Van den Berghe, G.; Verwaest, C.; Lauwers, P. Removal of pro-inflammatory cytokines with renal replacement therapy: sense or nonsense? Intensive Care Med. **1995**, *21* (2), 169–176.
5. De Vriese, A.S.; Colardyn, F.A.; Philippe, J.J.; Vanholder, R.C.; DeSutter, J.H.; Lameire, N.H. Cytokine removal during continuous hemofiltration in septic patients. J. Am. Soc. Nephrol. **1999**, *10* (4), 846–853.
6. Kline, J.A.; Gordon, B.E.; Williams, C.; Blumenthal, S.; Watts, J.A.; Diaz-Buxo, J. Large-pore hemodialysis in acute endotoxin shock. Crit. Care Med. **1999**, *27* (3), 588–596.
7. American College of Chest Physicians/ Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit. Care Med. **1992**, *20* (6), 864–874.
8. Thadhani, R.; Pascual, M.; Bonventre, J.V. Acute renal failure. N. Engl. J. Med. **1996**, *334* (22), 1448–1460.
9. Knaus, W.A.; Draper, E.A.; Wagner, D.P.; Zimmerman, J.E. APACHE II: a severity of disease classification system. Crit. Care Med. **1985**, *13* (10), 818–829.
10. Nakamura, H.; Kim, D.K.; Philbin, D.M.; Peterson, M.B.; Debros, F.; Koski, G.; Bonventre, J.V. Heparin-enhanced plasma phospholipase $A_2$ activity and prostacyclin synthesis in patients undergoing cardiac surgery. J. Clin. Invest. **1995**, *95* (3), 1062–1070.
11. Murakami, M.; Shimbara, S.; Kambe, T.; Kuwata, H.; Winstead, M.V.; Tischfield, J.A.; Kudo, I. The functions of five distinct mammalian phospholipase $A_2$s in regulating arachidonic acid release. J. Biol. Chem. **1998**, *273* (23), 14411–14423.
12. Song, C.H.; Choi, J.S.; Kim, D.K.; Kim, J.C. Enhanced secretory group II $PLA_2$ activity in the tears of chronic blepharitis patients. Invest. Ophthalmol. Vis. Sci. **1999**, *40* (11), 2744–2748.
13. Murakami, M.; Kudo, I.; Inoue, K. In vivo release and clearance of rat platelet phospholipase $A_2$. Biochim. Biophys. Acta **1989**, *1005* (3), 270–276.
14. Yekebas, E.F.; Treede, H.; Knoefel, W.T.; Bloechle, C.; Fink, E.; Izbicki, J.R. Influence of zero-balanced hemofiltration on the course of severe experimental pancreatitis in pigs. Ann. Surg. **1999**, *229* (4), 514–522.
15. Katayama, H. Comparison of continuous renal replacement therapy by clearance measurement. Journal of the Japanese Society of Intensive Care Medicine **1998**, *5* (2), 115–121.




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