Extracorporeal CO2 Removal May Improve Renal Function of Patients w ith ARDS and Acute K idney Inj ury

EXTRACORPOREAL CO_2 REMOVAL IMPROVES RENAL FUNCTION OF PATIENTS WITH ARDS AND ACUTE KIDNEY INJURY

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Running Title: ECCO$_2$R in patients with AKI and ARDS

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VF: Study design, data analysis and writing up of the first draft of the paper; VC: Study design and writing the manuscript; FA: Patient recruitment, data analysis and manuscript revision; GR: Patient recruitment and data analysis manuscript revision; AC: Patient recruitment, data collection and manuscript revision; CR: Data collection and analysis, revision of the manuscript; PC: Data collection and analysis, manuscript revision; CM: Patient recruitment, data collection and writing up of the first draft of the paper; CF: Data analysis and manuscript revision; LBr: Study design and manuscript revision; FP: Data collection and manuscript revision; LBi: Study design and manuscript revision; PT: Patient recruitment, study design and manuscript revision; LM: Data analysis and manuscript revision; VMR: Study design patient recruitment, data collection and analysis, and writing the manuscript; All the authors approved the final version to be published and agreed to be accountable for all aspects of the work.

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At a Glance Commentary

Scientific Knowledge on the Subject

In patients with ARDS, super-protective ventilation with tidal volume lower than 6 ml/Kg may further minimize ventilator induced lung injury but extracorporeal CO_2 removal needs be implemented to manage respiratory acidosis. Renal replacement therapy may incorporate extracorporeal CO_2 removal to support simultaneously lung and kidney functions. Data about biological determinants and clinical effects of this the simultaneous support of kidney and lung dysfunction are lacking.

What This Study Adds to the Field

Extracorporeal CO_2 removal may safely be integrated to conventional renal replacement therapy to allow super-protective ventilation may safely enhancing recovery of renal function in patients with ARDS and AKI. This effect seems to be associated with a reduction of concentration of inflammatory mediators and attenuation of plasmatic pro-apoptotic activity.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Abstract

Rationale. Combined supportive therapy of renal replacement therapy (RRT) and extra corporeal CO_2 removal ($ECCO_2R$) is proposed for patients with acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI) patients to further reduce tidal stress and strain.

Objectives. To determine whether ultra-protective ventilation during RRT and $ECCO_2R$ preserves renal function through attenuation of inflammatory and apoptotic processes.

Methods. Mechanically ventilated ARDS patients on RRT were included. In ***"RRT-plus-$ECCO_2R$"***, we aimed to reduce tidal volume to 4 ml/kg. A control group was created from ARDS patients on RRT ventilated with conventional tidal volume enrolled in previous studies (***"RRT-only"***). End-points were reduction in creatinine levels, days on renal replacement therapy, trans-epithelial electrical resistance. Cell expression of apoptosis-related molecules and concentration of interleukin-6 were evaluated on plasma collected at baseline (T0) and every 24 hours (T1, T2, T3). Previously defined ***"RRT-plus-$ECCO_2R$"*** related side-effects were recorded.

Measurements and Main Results. Matching algorithm identified thirteen patients for comparisons. In the ***"RRT-plus-$ECCO_2R$"***, despite tidal volume was reduced from 7.2±0.5 ml/kg to 5.1±0.7 ($p<0.05$), $PaCO_2$ and pH remained stable. Creatinine reduction was larger and days on renal replacement therapy were smaller in ***"RRT-plus-$ECCO_2R$"*** than ***"RRT-only"*** (49±20 vs. 26±22% and 7±3 vs. 10±4 days, respectively $p<0.05$). Interleukin-6 decreased over time, trans-epithelial electrical resistance returned to values of healthy controls and expression of apoptosis significantly decreased only in the ***"RRT-plus-$ECCO_2R$"***. No adverse events were reported.

Conclusions. ***"RRT-plus-$ECCO_2R$"*** safely allows ultra-protective ventilation that enhances recovery of renal function, and attenuates inflammatory and apoptotic processes.

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Key words: Mechanical ventilation, renal replacement therapy, extracorporeal life support, apoptosis, cytokines.

Introduction

Translocation of inflammatory mediators from the lungs to the systemic circulation and activation of pro-apoptotic pathways are the mechanisms advocated to explain end-organs failure consequent to ventilator induced lung injury (1). The efficacy of protective ventilation to improve outcome has been associated to the reduction of the systemic inflammatory response (2, 3) and the attenuation of the pro-apoptotic response of end-organs (1).

It has been recently shown that conventional protective ventilator settings [tidal volume (V_T) of 6 ml/kg and plateau pressure (P_{PLAT}) of 30 cmH_2O] may not always protect from ventilator induced lung injury (4, 5). Further reduction of V_T to 4 ml/kg and P_{PLAT} to 25 cmH_2O may attenuate the pulmonary and systemic inflammatory response (6), and may improve clinical outcome (7). These "super-protective" ventilator settings need to be integrated by extra-corporeal CO_2 removal ($ECCO_2R$) to manage the respiratory acidosis consequent to low minute ventilation (8).

Mechanical ventilation is a risk factor independently associated to mortality in patients with acute kidney injury (AKI) (9). In these patients, plasma concentration of inflammatory biomarkers predicts the development of AKI (10), and the lack of recovery of kidney function (11). Moreover, apoptosis of renal tubular cells has been advocated to explain the relationship between increased inflammatory response and occurrence of AKI (12-14).

Recent studies showed that renal replacement therapy (RRT) and $ECCO_2R$ may be combined in a single treatment to allow "super-protective" settings in patients with acute respiratory distress (ARDS) who suffer of AKI (15, 16). However, comparisons between ***"RRT-plus-ECCO$_2$R"*** with ultra-protective ventilation and ***"RRT-only"*** with conventional ventilator settings are not available.

The present study set up to examine the hypothesis that adding $ECCO_2R$ to conventional RRT may allow "super-protective" ventilation and thus improve markers of

renal function by reducing the plasmatic concentration of inflammatory mediators and decreasing the pro-apoptotic insult on renal tubular epithelial cells in patients requiring mechanical ventilation for ARDS and RRT for AKI.

Methods

The study included patients older than 18 and younger than 90 years who required mechanical ventilation for ARDS (17) and RRT for AKI (18,19). Patients were enrolled in three Italian intensive care units in the period December 2015-March 2017 (**ClinicalTrials.gov Identifier:** NCT02595619). Review boards approved the protocol (20). Detailed description of methods is given in on line data supplement.

"RRT-plus-ECCO$_2$R"

Patients were ventilated according to the ARDSnet protective strategy (21). RRT was performed using a continuous veno-venous hemodiafiltration (CVVHDF) mode. A polipropilene hollow fiber membrane lung was inserted in series before the hemofilter (Diapact CRRT®; B.Braun, Melsungen, Germany).

Anticoagulation was ensured by continuous infusion of heparin (8). In patients who had suspected or confirmed heparin induced thrombocytopenia or had contraindication for systemic anticoagulation, a calcium-free citrate replacement fluid was used **(Table E1, on line supplement)** (22,23).

The RRT was commenced at a blood flow rate of 300 ml/min and a sweep gas of 0 L/min (time 0, T0). V_T was hence gradually reduced targeting a minimum value of 4 ml/kg by 0.5 ml/kg while increasing PEEP to target a P_{PLAT} of 25 cmH_2O (8). Once the lowest value of V_T was reached, sweep gas (100% oxygen) was switched on at 10 L/min to obtain a value of $PaCO_2$ similar to baseline (±20%) with a pH >7.30 and respiratory rate (RR) ≤35/min (8). Decision to interrupt RRT was left to the attending clinician using standard criteria (24).

"RRT-only"

A dataset for matched cohort analysis was created using data from ARDS patients enrolled in previous studies (5,25) and ventilated according to a conventional protective strategy (21). RRT was performed using a continuous veno-venous hemofiltration (CVVH)

mode (Lynda CRRT®; BELLCO, Mirandola, Italy). Patients were considered for matching if required RRT for AKI using the same criteria as for the ***"RRT-plus-ECCO$_2$R"*** group.

In the ***"RRT-plus-ECCO2R"*** group, clinical data and plasma samples were collected at T0, and 24 (T1), 48 (T2) and 72 (T3) hours after reduction of V_T. In the ***"RRT-only"*** group, data analysis was performed using values collected at same time points. Creatinine concentrations until RRT was interrupted, days on RRT, hospital mortality and mortality at 28 days were recorded (24). Potential adverse events related to ***"RRT-plus-ECCO$_2$R"*** were recorded (6,26).

Assay for plasmatic concentration of interleukin-6 was carried out using a solid-phase enzyme-linked immunosorbent assay method. Alteration of cell permeability and polarity was assessed measuring trans-epithelial electrical resistance (TEER) (27). Expression of apoptosis related proteins was assessed through Proteome Profiler Human Apoptosis Array kit (R&D Systems, Space Import-Export, Milano, MI, Italy). All measurements were performed by technicians blinded to the study group.

Each patient treated with ***"RRT-plus-ECCO$_2$R"*** was matched with a patient treated with ***"RRT-only"*** with the closest propensity score selected using a Greedy algorithm. The balance statistics was performed using the Wilcoxon-Mann-Whitney test. Values obtained at the different times were compared using analysis of variance for repeated measure and a post hoc Dunnett test. Outcomes before and after matching were compared between treatment groups with Chi-square test or Wilcoxon-Mann-Whitney test as appropriate.

Results

Eighty-five mechanically ventilated ARDS patients were admitted. Fourteen patients were treated with ***"RRT-plus-ECCO$_2$R"*** and were eligible for matching with the control group. The remaining 71 patients were excluded for not having AKI (N=50), for having severe ARDS (N=16), for being treated with RRT before ICU admission (N=4), and for limiting therapeutic intervention (N=1). Of the 183 patients with ARDS enrolled in previous studies (6, 25), 40 patients were treated with ***"RRT-only"*** and were eligible for matching. Remaining patients were excluded for not having AKI (N=62), for having severe ARDS (N=23), for being treated with RRT before ICU admission (N=4), and for limiting therapeutic intervention (N=3). Thirty-eight patients were also excluded since biological samples were not available. The propensity score matching and the Greedy algorithm identified 13 patients from the ***"RRT-plus-ECCO$_2$R"*** group to be compared with 13 patients from the ***"RRT-only"*** group (**Figure 1**) enrolled in previous studies (6,25).

Before matching, SAPS II, and days of mechanical ventilation before the initiation of RRT were higher in the ***"RRT-plus-ECCO$_2$R"*** group than in the ***"RRT-only"*** group (p=0.048 and 0.03, respectively). After matching, all baseline characteristics were similar in the two groups (**Table 1**). Further clinical characteristics of patients in the two study groups are given in the on-line supplement (**Table E2** on-line supplement).

Physiological variables and ventilator settings of patients in the two study groups are reported in **Figure 2**. In the ***"RRT-plus-ECCO2R"*** group, V_T was reduced to approximately 5 ml/kg PBW while $PaCO_2$ and pH remained stable. Concomitantly, despite an almost 20% increase of PEEP, P_{PLAT} and driving pressure were significantly lower at T1, T2 and T3 than at T0 ($p<0.05$). In patients treated with ***"RRT-only"***, values of V_T remained in the range of 6-8 ml/kg PBW, and $PaCO_2$, pH, PEEP, P_{PLAT}, and driving pressure remained stable. PaO_2/FiO_2 ratio did not change in the two groups throughout the study period (**Table E3** on-line supplement).

The mean arterial pressure, effluent flow rate and the amount of fluid removal did not differ between the two groups. Blood flow was 30-53% higher in ***"RRT-plus-ECCO$_2$R"*** than in ***"RRT-only"*** throughout the study period (276±53 vs. 200±8, 286±46 vs. 220±10, 318±25 vs. 220±10, 317±28 vs 180±30 in ***"RRT-plus-ECCO$_2$R"*** than in ***"RRT-only"*** at T=, T1, T2 and T3, respectively; $p<0.05$). In the ***"RRT-plus-ECCO$_2$R"*** group six patients received heparin and seven received citrate, while all patients treated with ***"RRT-only"*** received heparin. Amount of heparin infused during ***"RRT-plus-ECCO$_2$R"*** was significantly higher than during ***"RRT-only"*** (14 vs 3 UI/kg/h; $p<0.05$). Operational characteristics of RRT and data on the heparin vs. citrate RRT settings in the two study groups are presented in in the on-line supplement (**Table E4** on-line supplement).

Clinical outcome variables are shown in **Table 2**. The reduction in creatinine from the time of initiation to the time of interruption of RRT was larger and the number of days of RRT was smaller in patients treated with ***"RRT-plus-ECCO$_2$R"*** than in patients treated with ***"RRT-only"*** ($p<0.05$). In patients of the ***"RRT-plus-ECCO$_2$R"*** group, extra-corporeal CO_2 removal was implemented for the 70±29% of total dialysis time (5±2 of days of the 7±3 days of dialysis). Mortality at 28 days and hospital mortality were lower in patients treated with ***"RRT-plus-ECCO$_2$R"*** than in patients treated with ***"RRT-only"*** although not significantly. No adverse events were reported during the study period.

Plasma concentration of IL6 at baseline did not differ between ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"*** and (a) significantly decreased over time in patients treated with ***"RRT-plus-ECCO$_2$R"*** while remained constant in patients treated with ***"RRT-only"***; (b) was significantly lower in ***"RRT-plus-ECCO$_2$R"*** than ***"RRT-only"*** at T1, T2, and T3 ($p<0.05$) (**Figure 3, panel A**). Renal tubular epithelial cells incubated with plasma collected at T0 from patients of the ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"*** groups,

exhibited TEER values similarly lower than those observed in renal tubular epithelial cells incubated with plasma from healthy controls and vehicle. After 72 hours of RRT, TEER values returned to values like those observed in healthy controls only in the ***"RRT-plus-$ECCO_2R$"*** group while remained significantly lower than those observed in healthy controls in the ***"RRT-only"*** group (**Figure 3, panel B**). Expression of cell receptors and of intracellular signaling molecules involved in apoptosis of renal tubular epithelial cells at T0 were similar in ***"RRT-plus-$ECCO_2R$"*** and ***"RRT-only"*** groups (**Figure 2, panel C and D**). Compared with T0, the expression of apoptosis related proteins significantly decreased at T3 in the ***"RRT-plus-$ECCO_2R$"***. In addition, compared to T0, the expression of intracellular signaling molecules significantly increased at T3 in the ***"RRT-only"*** group (**Figure 2, panel C and D**). Representative images related to the expression level of apoptosis-related proteins are given in **Figure 4** and **Figure 5**.

Discussion

The present study shows that RRT and $ECCO_2R$ may be performed simultaneously in ARDS patients that develop AKI allowing the use of super-protective ventilation while supporting renal function. Recovery of renal function was more pronounced and concentration of inflammatory and pro-apoptotic mediators was lower when super-protective ventilation was allowed by ***"RRT-plus-$ECCO_2R$"*** than when conventional ventilator settings were used during ***"RRT-only"***. Differently from a previous study (6), no mechanical nor patient-related adverse events were reported during the use of ***"RRT-plus-$ECCO_2R$"***. Of interest, our study shows that citrate regional anticoagulation may be used in the context of ***"RRT-plus-$ECCO_2R$"*** for those patients who had contraindications to systemic anticoagulation with heparin. With a blood flow up to 350 ml/min, no perturbation of calcium homeostasis was reported with a continuous infusion of calcium chloride ranging between 4 and 7 mEq/h. Citrate was dissolved in the replacement solution and the effluent flow rate was adapted to blood flow according to a predefined protocol (22, 23). Recently, Faguer et al showed that calcium free and citrate containing replacement fluid was effective and safe during intermittent hemodialysis with very low incidence of circuit clotting (28).

Mechanical ventilation, one of the strongest predictor of mortality in patients with AKI (9, 29) is known to expose patients to ventilator induced lung injury (1). Lung protective ventilation is thought to improve outcome by reducing the plasmatic concentration of inflammatory and apoptotic factors and attenuating cellular apoptosis in end-organs such as the kidney and the small intestine (30). Recent data show that a further reduction of V_T to 3-4 ml/kg and P_{PLAT} to 25 cmH_2O results in an enhanced attenuation in pulmonary inflammation (6) and in the reduction in days spent on mechanical ventilation (7). In all these studies, the respiratory acidosis derived from the reduction of minute ventilation was managed by $ECCO_2R$ (31). These data, together with the increase perception of the clinical relevance of inter-organ cross-talk between the kidneys and the lungs (32) led to the development of

extracorporeal circuits for RRT incorporating a membrane oxygenator to provide super-protective mechanical ventilation for patients that concomitantly suffer of renal and respiratory failure (15,16). In two series of patients with both ARDS and AKI, a membrane oxygenator (0.65-0.67 m^2) upstream of the hemofilter (1.4-1.5 m^2) reduced $PaCO_2$ of 20-30% of baseline with a blood flow of 400 ml/min, thus allowing further V_T reduction without any major adverse events. Forster and coworkers showed that this combined strategy was associated with lower need of vasopressor (16). However, up to the present study, no data are available on the comparison in terms of safety and efficacy between ***"RRT-plus-ECCO$_2$R"*** with the ventilator set with "super-protective" settings and ***"RRT-only"*** with conventional protective ventilation.

The strength of this study lies in the fact that the homogeneity of patients included in the study and the robustness of the matching method, allowed a comparison between ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"***. We identified variables such as age, severity of critical illness disease (SAPS II), severity of AKI (KIDGO) and duration of dependence form mechanical ventilation (days of mechanical ventilation) to make the two groups as much similar as possible to compare. Moreover, the reported risk of atelectasis and hypoxemia during $ECCO_2R$ due to a decrease in mean airway pressure was prevented in the present study by a 20% increase in PEEP (from 9.4±3.7 cmH_2O at T0 to 10.8±3.7, 10.6±2.6 and 11.3±3.8 cmH_2O at T1, T2, and T3, respectively $p<0.05$) that maintained PaO_2/FiO_2 unchanged throughout the study period.

Caution must be exercised in generalizing results of the present study since several limitations need to be addressed. First, as in a previous study (26), institutional review boards refused to authorize a randomized clinical trial due to the limited amount of information regarding the use of ***"RRT-plus-ECCO$_2$R"***. Although appropriate design of matched cohort study may minimize the risk of systematic overestimation of the magnitude of the treatment, the non-randomized design remains the major limitation. To minimize

overestimation of the treatment effect, (a) we assessed recovery of renal function using objective measurement used in large previous studies such as the changes in creatinine with RRT and the number of days on RRT (24); (b) the reabsorption function of tubular cells, responsible for maintaining volume balance and fluid composition was assessed by measurement of TEER, a well-established method for monitoring the alteration of selective cell permeability and polarity integrity (27); (c) we used objective and duplicated measurement of biological samples performed by technicians blinded to the study group. Second, two different RRT modes such as CVVHDF and CVVH were used in the "***RRT-plus-ECCO$_2$R***" and "***RRT-only***" groups, respectively. However, it is unlikely that the diffusion modality used in the "***RRT-plus-ECCO$_2$R*** affected our clinical and experimental findings. In fact, inflammatory mediators are middle molecules that are better removed by convective/mixed techniques. Moreover, the prescribed RRT dose did not differ between the two study groups (33).

In critically ill patients with AKI requiring RRT, increased plasma concentrations of inflammatory biomarkers have been associated with RRT dependence and risk of death (11). In particular, higher concentrations of IL6 predict either the development of AKI (10) and the risk of death (34). Recent evidences highlight the importance of apoptosis in the pathogenesis of AKI. Circulating factors derived from plasma of critically ill patients have the potential of inducing renal cell apoptosis (13,14,35) activating caspases and initiating apoptosis (36). This process is mediated by both extrinsic apoptotic pathway activated by surface receptors including Fas on renal tubular cells (37,38), and activation of intra-cellular pathways that through the increase in Bax/Bcl2 ratio promote mitochondrial membrane permeability and apoptosis (39). Our data show that, expression of cell receptors and intracellular signaling molecules involved in apoptosis of renal tubular epithelial cells was attenuated when super-protective ventilation was allowed by ***"RRT-plus-ECCO$_2$R"***.

In conclusion, this study provides evidence that combining in a single treatment ECCO$_2$R and RRT in patients requiring mechanical ventilation for ARDS and renal support

for AKI safely allows the use of super-protective mechanical ventilation that results in a recovery of renal function, in a reduction of concentration of inflammatory mediators, and in an attenuation of plasmatic pro-apoptotic activity that is more pronounced than when RRT is delivered while ventilating patients with conventional settings. These findings allow us to speculate that $ECCO_2R$ might be actively incorporated in RRT circuits and provide the rationale for future randomized clinical trials.

Bibliography

1. Slutsky AS, Ranieri VM. Ventilator-induced lung injury. *The New England Journal of Medicine* 2013;369:2126-2136.
2. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, Wheeler AP. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005;33:1-6; discussion 230-232.
3. Acute Respiratory Distress Syndrome N, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342:1301-1308.
4. Bellani G, Guerra L, Musch G, Zanella A, Patroniti N, Mauri T, Messa C, Pesenti A. Lung regional metabolic activity and gas volume changes induced by tidal ventilation in patients with acute lung injury. *Am J Respir Crit Care Med* 2011;183:1193-1199.
5. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, Gandini G, Herrmann P, Mascia L, Quintel M, Slutsky AS, Gattinoni L, Ranieri VM. Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2007;175:160-166.
6. Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, Faggiano C, Quintel M, Gattinoni L, Ranieri VM. Tidal volume lower than 6 ml/kg enhances lung protection: Role of extracorporeal carbon dioxide removal. *Anesthesiology* 2009;111:826-835.
7. Bein T, Weber-Carstens S, Goldmann A, Muller T, Staudinger T, Brederlau J, Muellenbach R, Dembinski R, Graf BM, Wewalka M, Philipp A, Wernecke KD, Lubnow M, Slutsky AS. Lower tidal volume strategy (approximately 3 ml/kg) combined with extracorporeal co2 removal versus 'conventional' protective ventilation (6 ml/kg) in severe ards : The prospective randomized xtravent-study. *Intensive Care Medicine* 2013;39:847-856.
8. Fanelli V, Ranieri MV, Mancebo J, Moerer O, Quintel M, Morley S, Moran I, Parrilla F, Costamagna A, Gaudiosi M, Combes A. Feasibility and safety of low-flow extracorporeal carbon dioxide removal to facilitate ultra-protective ventilation in patients with moderate acute respiratory distress sindrome. *Crit Care* 2016;20:36.
9. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C. Acute renal failure in critically ill patients: A multinational, multicenter study. *JAMA : the journal of the American Medical Association* 2005;294:813-818.
10. Chawla LS, Seneff MG, Nelson DR, Williams M, Levy H, Kimmel PL, Macias WL. Elevated plasma concentrations of il-6 and elevated apache ii score predict acute kidney injury in patients with severe sepsis. *Clin J Am Soc Nephrol* 2007;2:22-30.
11. Murugan R, Wen X, Shah N, Lee M, Kong L, Pike F, Keener C, Unruh M, Finkel K, Vijayan A, Palevsky PM, Paganini E, Carter M, Elder M, Kellum JA, for the Biological Markers for Recovery of Kidney Study I. Plasma inflammatory and apoptosis markers are associated with dialysis dependence and death among critically ill patients receiving renal replacement therapy. *Nephrol Dial Transplant* 2014.
12. Jo SK, Cha DR, Cho WY, Kim HK, Chang KH, Yun SY, Won NH. Inflammatory cytokines and lipopolysaccharide induce fas-mediated apoptosis in renal tubular cells. *Nephron* 2002;91:406-415.
13. Guo R, Wang Y, Minto AW, Quigg RJ, Cunningham PN. Acute renal failure in endotoxemia is dependent on caspase activation. *Journal of the American Society of Nephrology : JASN* 2004;15:3093-3102.
14. Mariano F, Cantaluppi V, Stella M, Romanazzi GM, Assenzio B, Cairo M, Biancone L, Triolo G, Ranieri VM, Camussi G. Circulating plasma factors induce tubular and glomerular alterations in septic burns patients. *Crit Care* 2008;12:R42.
15. Allardet-Servent J, Castanier M, Signouret T, Soundaravelou R, Lepidi A, Seghboyan JM. Safety and efficacy of combined extracorporeal co2 removal and renal replacement therapy in patients with acute respiratory distress syndrome and acute kidney injury: The pulmonary and renal support in acute respiratory distress syndrome study. *Crit Care Med* 2015;43:2570-2581.
16. Forster C, Schriewer J, John S, Eckardt KU, Willam C. Low-flow co(2) removal integrated into a renal-replacement circuit can reduce acidosis and decrease vasopressor requirements. *Crit Care* 2013;17:R154.

17. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: The berlin definition. *JAMA : the journal of the American Medical Association* 2012;307:2526-2533.
18. Kidgo aki guidline. 2012.
19. Tolwani A. Continuous renal-replacement therapy for acute kidney injury. *The New England Journal of Medicine* 2012.
20. Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, Finfer S, Gardlund B, Marshall JC, Rhodes A, Artigas A, Payen D, Tenhunen J, Al-Khalidi HR, Thompson V, Janes J, Macias WL, Vangerow B, Williams MD. Drotrecogin alfa (activated) in adults with septic shock. *The New England journal of medicine* 2012;366:2055-2064.
21. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The acute respiratory distress syndrome network. *N Engl J Med* 2000;342:1301-1308.
22. Nurmohamed SA, Vervloet MG, Girbes AR, Ter Wee PM, Groeneveld AB. Continuous venovenous hemofiltration with or without predilution regional citrate anticoagulation: A prospective study. *Blood Purif* 2007;25:316-323.
23. Aman J, Nurmohamed SA, Vervloet MG, Groeneveld AB. Metabolic effects of citrate- vs bicarbonate-based substitution fluid in continuous venovenous hemofiltration: A prospective sequential cohort study. *J Crit Care* 2010;25:120-127.
24. Network VNARFT, Palevsky PM, Zhang JH, O'Connor TZ, Chertow GM, Crowley ST, Choudhury D, Finkel K, Kellum JA, Paganini E, Schein RM, Smith MW, Swanson KM, Thompson BT, Vijayan A, Watnick S, Star RA, Peduzzi P. Intensity of renal support in critically ill patients with acute kidney injury. *N Engl J Med* 2008;359:7-20.
25. Terragni PP, Filippini C, Slutsky AS, Birocco A, Tenaglia T, Grasso S, Stripoli T, Pasero D, Urbino R, Fanelli V, Faggiano C, Mascia L, Ranieri VM. Accuracy of plateau pressure and stress index to identify injurious ventilation in patients with acute respiratory distress syndrome. *Anesthesiology* 2013;119:880-889.
26. Del Sorbo L, Pisani L, Filippini C, Fanelli V, Fasano L, Terragni P, Dell'Amore A, Urbino R, Mascia L, Evangelista A, Antro C, D'Amato R, Sucre MJ, Simonetti U, Persico P, Nava S, Ranieri VM. Extracorporeal co2 removal in hypercapnic patients at risk of noninvasive ventilation failure: A matched cohort study with historical control. *Crit Care Med* 2014.
27. Cantaluppi V, Assenzio B, Pasero D, Romanazzi GM, Pacitti A, Lanfranco G, Puntorieri V, Martin EL, Mascia L, Monti G, Casella G, Segoloni GP, Camussi G, Ranieri VM. Polymyxin-b hemoperfusion inactivates circulating proapoptotic factors. *Intensive Care Medicine* 2008;34:1638-1645.
28. Faguer S, Saint-Cricq M, Nogier MB, Labadens I, Lavayssiere L, Kamar N, Cointault O. Heparin-free prolonged intermittent hemodialysis using calcium-free citrate dialysate in critically ill patients. *Crit Care Med* 2017;45:1887-1892.
29. Liu KD, Matthay MA. Advances in critical care for the nephrologist: Acute lung injury/ards. *Clin J Am Soc Nephrol* 2008;3:578-586.
30. Ranieri VM, Giunta F, Suter PM, Slutsky AS. Mechanical ventilation as a mediator of multisystem organ failure in acute respiratory distress syndrome. *Jama* 2000;284:43-44.
31. Fanelli V, Costamagna A, Ranieri VM. Extracorporeal support for severe acute respiratory failure. *Semin Resp Crit Care* 2014;35:519-527.
32. Husain-Syed F, Slutsky AS, Ronco C. Lung-kidney crosstalk in the critically ill patient. *Am J Respir Crit Care Med* 2016.
33. Garzotto F, Ostermann M, Martin-Langerwerf D, Sanchez-Sanchez M, Teng J, Robert R, Marinho A, Herrera-Gutierrez ME, Mao HJ, Benavente D, Kipnis E, Lorenzin A, Marcelli D, Tetta C, Ronco C, DoRe Msg. The dose response multicentre investigation on fluid assessment (doremifa) in critically ill patients. *Crit Care* 2016;20:196.
34. Simmons EM, Himmelfarb J, Sezer MT, Chertow GM, Mehta RL, Paganini EP, Soroko S, Freedman S, Becker K, Spratt D, Shyr Y, Ikizler TA, Group PS. Plasma cytokine levels predict mortality in patients with acute renal failure. *Kidney Int* 2004;65:1357-1365.

35. Cantaluppi V, Weber V, Lauritano C, Figliolini F, Beltramo S, Biancone L, De Cal M, Cruz D, Ronco C, Segoloni GP, Tetta C, Camussi G. Protective effect of resin adsorption on septic plasma-induced tubular injury. *Crit Care* 2010;14:R4.
36. Ruchalski K, Mao H, Li Z, Wang Z, Gillers S, Wang Y, Mosser DD, Gabai V, Schwartz JH, Borkan SC. Distinct hsp70 domains mediate apoptosis-inducing factor release and nuclear accumulation. *The Journal of biological chemistry* 2006;281:7873-7880.
37. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ. Acute renal failure in endotoxemia is caused by tnf acting directly on tnf receptor-1 in kidney. *J Immunol* 2002;168:5817-5823.
38. Al-Lamki RS, Mayadas TN. Tnf receptors: Signaling pathways and contribution to renal dysfunction. *Kidney Int* 2015;87:281-296.
39. Havasi A, Li Z, Wang Z, Martin JL, Botla V, Ruchalski K, Schwartz JH, Borkan SC. Hsp27 inhibits bax activation and apoptosis via a phosphatidylinositol 3-kinase-dependent mechanism. *The Journal of biological chemistry* 2008;283:12305-12313.

Figure Legends

Figure 1. Patient's characteristics and selection criteria for inclusion in study analysis. ARDS: acute respiratory distress syndrome, AKI: acute kidney injury, RRT: renal replacement therapy, $ECCO_2R$: extracorporeal CO_2 removal.

Figure 2. Time course of ventilatory variables in ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"***. RRT: renal replacement therapy, $ECCO_2R$: extracorporeal CO_2 removal, V_T: tidal volume, $PaCO_2$ arterial partial pressure of carbon dioxide.

Figure 3. Time course of plasmatic concentration of interleukin 6 (IL6) in patients treated with ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"***. §p<.05 T3 vs. T0; *p<.05 ***"RRT-plus-ECCO$_2$R"*** vs ***"RRT-only"***. (**panel A**). Trans-epithelial resistance (TEER) of renal tubular cells after incubation with plasma from patients of "***RRT-only***" and ***"RRT-plus-ECCO$_2$R"*** groups. § p<.05 T3 vs T0 (**panel B**). Renal tubular epithelial cells expression of **tumor necrosis factor receptor 1 (TNFR1), Fas cluster of differentiation 95 (Fas/CD95)**, pro-caspase 3, cleaved caspase 3, **tumor necrosis factor receptor 1 (TRAIL R1), tumor necrosis factor receptor 2 (TRAIL R2)**. after stimulation with plasma obtained from patients treated with ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"*** at T0 and T3. °p<.05 RRT-only at T3 vs T0. *p<.05 ***"RRT-plus-ECCO$_2$R"*** at T3 vs T0 (**panel C**). Renal tubular epithelial cells expression of bcl-2-associated death promoter (**Bad**), bcl-2-associated protein X (**Bax**), pro-caspase 3, and cleaved caspase 3 after stimulation with plasma obtained from patients treated with ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"*** at T0 and at T3. *p<.05 RRT-plus-ECCO2R at T3 vs T0 (**panel D**).

Figure 4. Apoptotic array spots showing renal tubular epithelial cells expression of TNFRI, Fas/CD95, TRAILR1, TRAILR2 after incubation with plasma from patients in the ***"RRT-plus- ECCO$_2$R"*** and ***"RRT-only"*** groups at T0 and T3.

Figure 5. Apoptotic array spots showing renal tubular epithelial cells expression of Bad, Bax, procaspase-3 and caspase-3 after incubation with plasma from patients in the ***"RRT-plus- ECCO$_2$R"*** and ***"RRT-only"*** groups at T0 and T3.

Table 1. Baseline characteristics before and after matching.

	"RRT-plus-$ECCO_2R$" (n=14)	*"RRT-only"* (n=40)		*"RRT-plus-$ECCO_2R$"* (n=13)	*"RRT-only"* (n=13)	
	Before Matching		p	***After Matching***		p
Age (yrs)	57(22)	63(13)	0.621	60(20)	60(13)	0.607
SAPS II	54(12)	47(10)	0.048	53(12)	52(16)	0.938
KIDGO I n (%) II n (%) III n (%)	 0(0) 2(14) 12(86)	 0(0) 8(20) 32(80)	>.999	 0(0) 2(15) 11(85)	 0(0) 1(8) 12(92)	>.999
Days of MV before RRT	6.8(4.7)	11(3.5)	0.03	7.8(3.5)	7.4(4.4)	0.623
V_T T0 (ml/kg/PBW)	7.2(0.6)	7.0(0.6)	0.566	7.03(0.46)	7.06(0.58)	0.611
Driving Pressure (cmH_2O)	19.3(3.9)	18.4(2.6)	0.597	19.2(2.2)	19.2(2.2)	0.817

Definition of abbreviations. SAPS II: Simplified Acute Physiology Score; KIDGO: Kidney Disease Improving Global Outcome; MV: Mechanical Ventilation; RRT: Renal Replacement Therapy; $ECCO_2R$: Extracorporeal CO_2 Removal.

Table 2. Clinical outcome variables

Variables	*"RRT-plus-$ECCO_2R$"*	*"RRT-only"*	p
Reduction in creatinine* *, %*			
Before matching	48 ± 19	29 ±26	0.0215
After matching	49 ± 20	26 ± 22	0.0208
Days of dialysis, *mean ± SD*			
Before matching	6 ± 3	11 ± 11	0.0128
After matching	7 ± 3	10 ± 4	0.0034
Hospital mortality, *n (%)*			
Before matching	8 (57)	29 (73)	0.3282
After matching	7 (54)	9 (69)	0.4201
Mortality at 28 days, *n (%)*			
Before matching	8 (57)	27 (68)	0.5278
After matching	7 (54)	8 (62)	0.6914

Definition of abbreviations. RRT: Renal Replacement Therapy; $ECCO_2R$: Extracorporeal CO_2 Removal

* Reduction in creatinine was calculated as creatinine at time of initiation of RRT - creatinine at time of interruption of RRT divided creatinine at time of initiation of RRT and expressed as % of the creatinine value at time of initiation of RRT

Figure 1

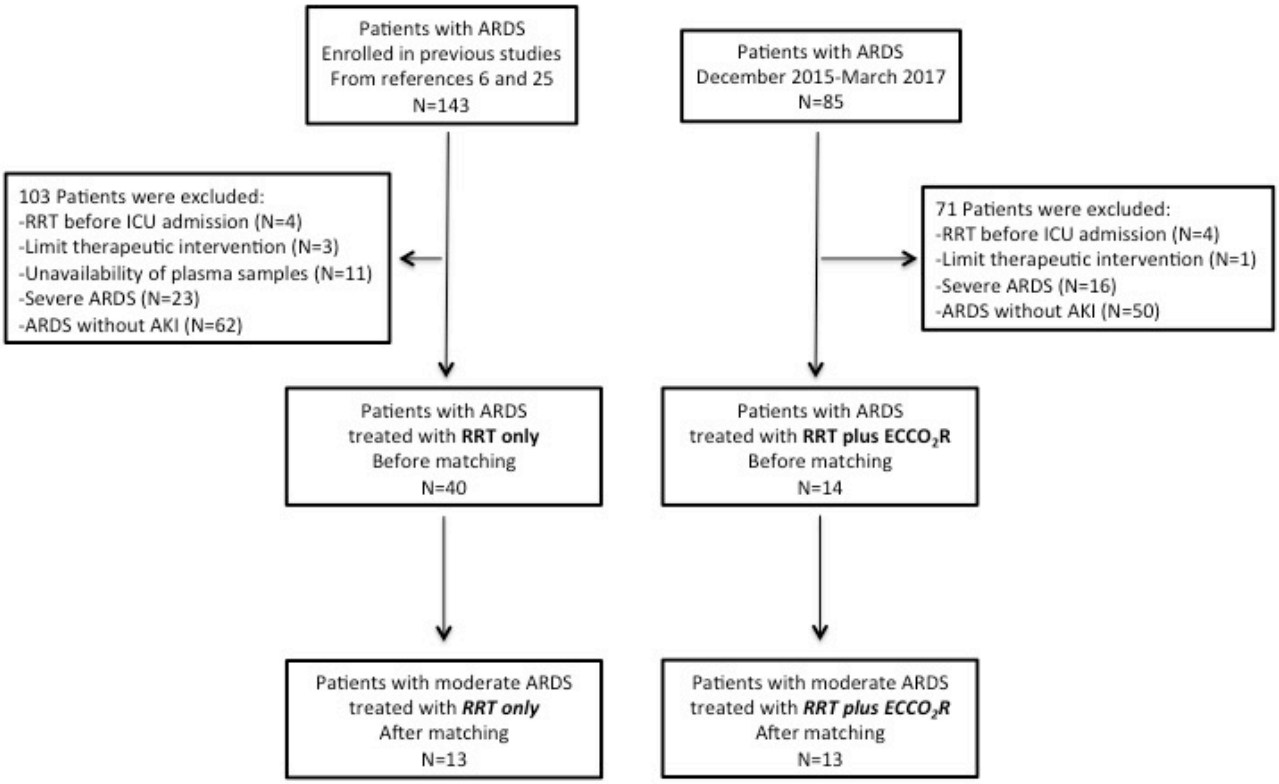

Patients with ARDS
Enrolled in previous studies
From references 6 and 25
N=143
Patients with ARDS
December 2015-March 2017
N=85
103 Patients were excluded:
-RRT before ICU admission (N=4)
-Limit therapeutic intervention (N=3)
-Unavailability of plasma samples (N=11)
-Severe ARDS (N=23)
-ARDS without AKI (N=62)
71 Patients were excluded:
-RRT before ICU admission (N=4)
-Limit therapeutic intervention (N=1)
-Severe ARDS (N=16)
-ARDS without AKI (N=50)
Patients with ARDS
treated with RRT only
Before matching
N=40
Patients with ARDS
treated with RRT plus ECCO2R
Before matching
N=14
Patients with moderate ARDS
treated with RRT only
After matching
N=13
Patients with moderate ARDS
treated with RRT plus ECCO2R
After matching
N=13

Figure 2

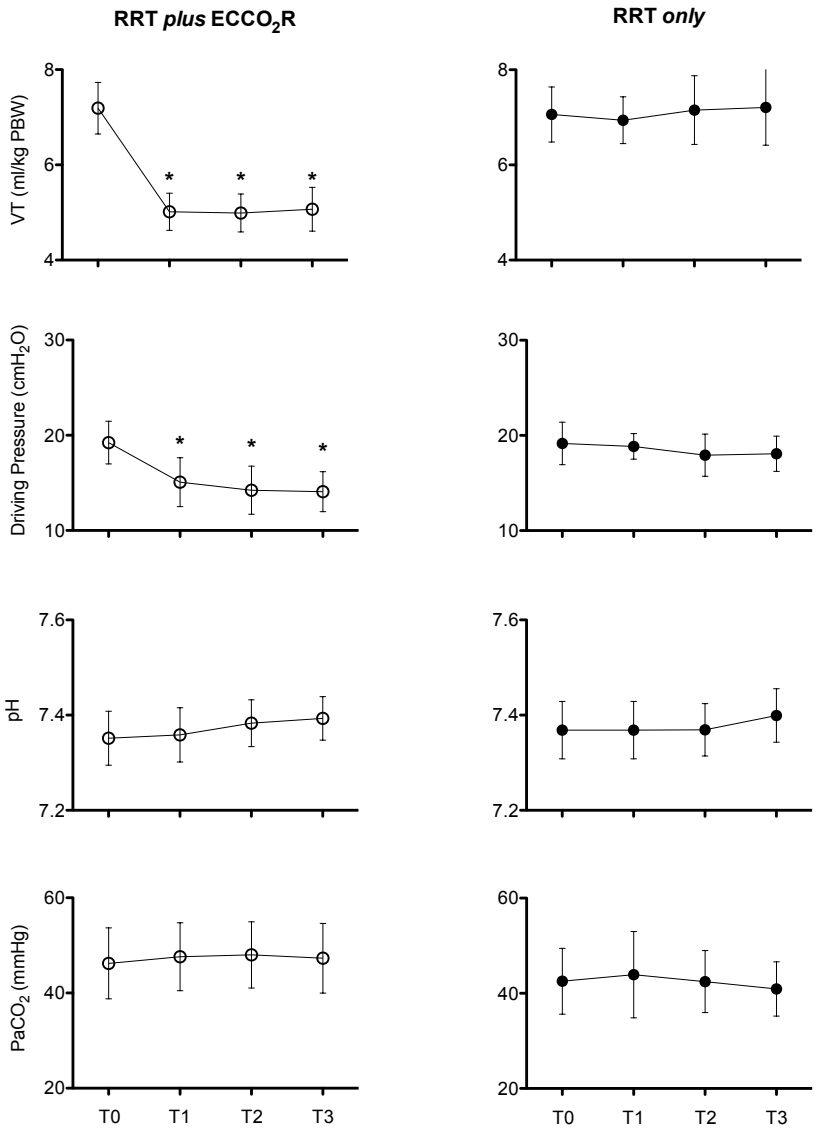

RRT *plus* ECCO2R
RRT *only*
VT (ml/kg PBW)
Driving Pressure (cmH2O)
pH
PaCO2 (mmHg)
T0
T1
T2
T3

Figure 3

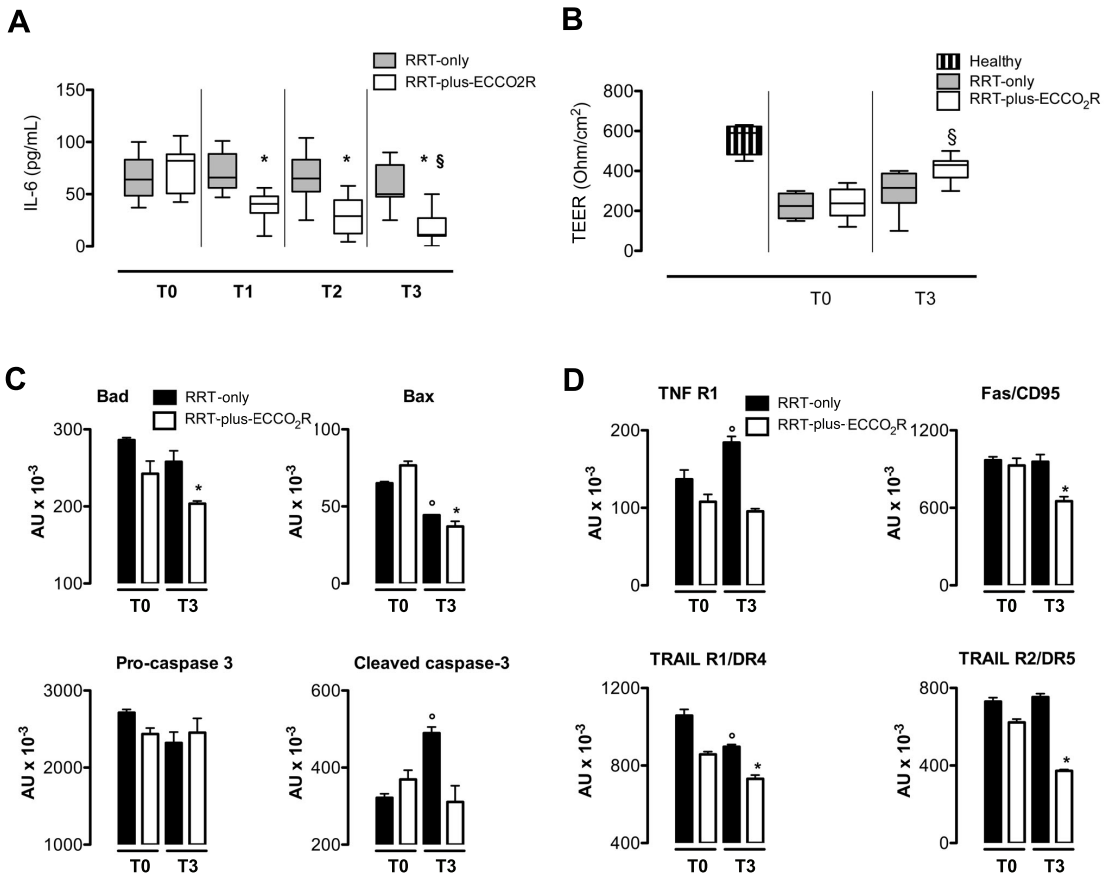

A
RRT-only
RRT-plus-ECCO2R
IL-6 (pg/mL)
150
100
50
0
T0
T1
T2
T3
B
Healthy
RRT-only
RRT-plus-ECCO2R
TEER (Ohm/cm2)
800
600
400
200
0
T0
T3
C
Bad
RRT-only
RRT-plus-ECCO2R
Bax
Pro-caspase 3
Cleaved caspase-3
AU x 10-3
D
TNF R1
RRT-only
RRT-plus-ECCO2R
Fas/CD95
TRAIL R1/DR4
TRAIL R2/DR5
AU x 10-3
T0
T3

Figure 4

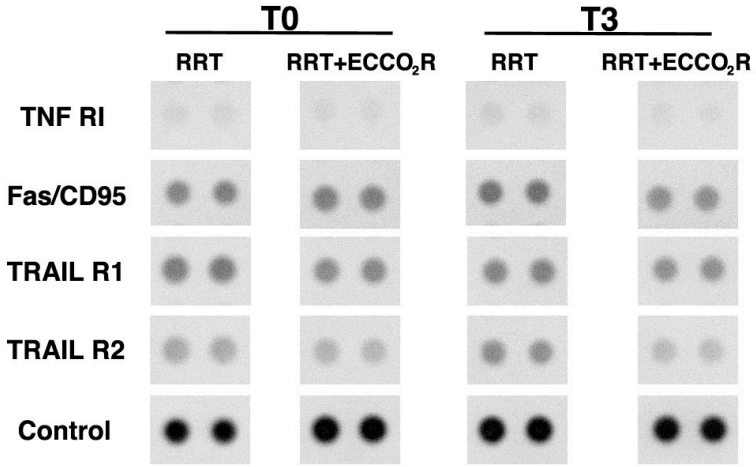
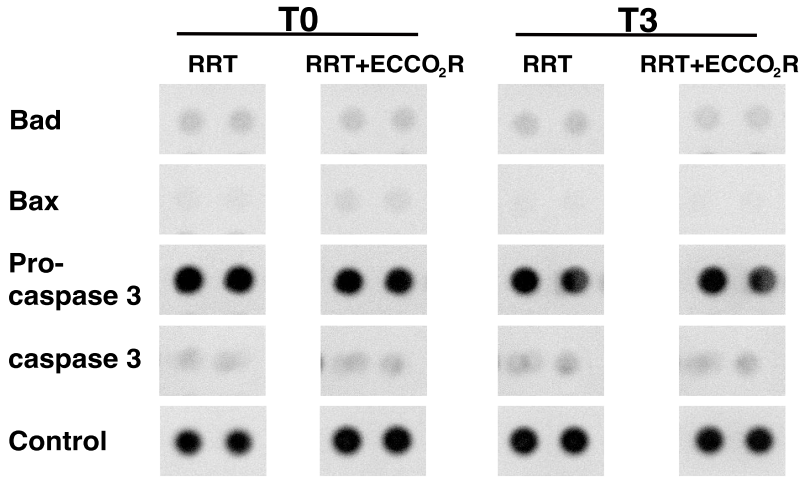

T0
T3
RRT
RRT+ECCO2R
RRT
RRT+ECCO2R
TNF RI
Fas/CD95
TRAIL R1
TRAIL R2
Control

Figure 5


T0
T3
RRT
RRT+ECCO2R
RRT
RRT+ECCO2R
Bad
Bax
Pro-
caspase 3
caspase 3
Control

EXTRACORPOREAL CO_2 REMOVAL IMPROVES RENAL FUNCTION OF PATIENTS WITH ARDS AND ACUTE KIDNEY INJURY

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Methods

The study included patients older than 18 and younger than 90 years who required mechanical ventilation for ARDS (1) and RRT for AKI (2, 3). Exclusion criteria were: severe ARDS; use of non-invasive ventilation; pregnancy; decompensated heart insufficiency or acute coronary syndrome; severe chronic obstructive pulmonary disease; respiratory acidosis with arterial PCO_2 ($PaCO_2$) >80 mmHg; acute brain injury; severe liver insufficiency (Child-Pugh score >7) or fulminant hepatic failure; patient moribund; decision to limit therapeutic interventions; need of renal replacement therapy before ICU admission. Patients were enrolled in three Italian intensive care units in the period December 2015-March 2017 (**ClinicalTrials.gov Identifier:** NCT02595619). Review boards approved the protocol. Because the patients were incompetent, consent was obtained in accordance with local ethics committee procedures (4).

"RRT-plus-ECCO$_2$R"

Patients were ventilated according to the ARDSnet protective ventilatory strategy (5). RRT was performed with Diapact CRRT® using a continuous veno-venous hemodiafiltration (CVVHDF) mode, equipped with a 1.5 m^2 polisulfon hemofilter (B.Braun Avitum, Melsungen, Germany). Blood flow was increased up to 350 ml/min with a prescribed dose of 45 ml/kg/hr (post-dilution mode 100%) and maintaining a filtration fraction lower than 25%. A polipropilene hollow fiber membrane lung (Euroset, Medolla, Italy; priming volume, 100 mL; contact surface area, 1.35 m^2) was inserted in series before the hemofilter. Percutaneous venous femoral or jugular cannulation was performed through insertion of a single dual-lumen catheter (14 Fr) (14F; Joline GmbH & Co. KG).

Anticoagulation was ensured by continuous infusion of heparin at 18 IU/kg/hr to maintain the activated partial thromboplastin time ratio between 1.50 and 2.34 without initial bolus and after

priming the system with 2 liters of normal saline containing 10000 IU of heparin (6). In patients who had suspected or confirmed heparin induced thrombocytopenia or had contraindication for systemic anticoagulation (any of the following: platelet count <30,000/mm^3; stroke or severe head trauma or intracranial arteriovenous malformation, cerebral aneurysm, CNS mass lesion within the previous 3 months, epidural catheter in place or expected to be positioned during the study, history of congenital bleeding diatheses, gastrointestinal bleeding within the 6 weeks prior to study entry, esophageal varices, chronic jaundice, cirrhosis, recent trauma), a calcium-free citrate replacement fluid (Dirinco AG, Bern 25, CH-3000 Switzerland; citrate concentration of 13.3 mmol/L) with a pre-dilution rate of 100% was used (7, 8). Calcium chloride was systemically administered through a venous central line to maintain calcium ionized between 1.0 and 1.1 mmol/L, in accordance with a predefined protocol **(Table 1, on line supplement)** (7, 8).

The RRT was commenced at a blood flow rate of 300 ml/min and a sweep gas of 0 L/min such that no CO_2 removal was performed (**time 0, T0**). V_T was hence gradually reduced targeting a minimum value of 4 ml/kg by 0.5 ml/kg while increasing PEEP to target a P_{PLAT} of 25 cmH_2O (6). Once the lowest value of V_T was reached, sweep gas (100% oxygen) was switched on at 10 L/min to obtain a value of $PaCO_2$ similar to baseline (±20%) with a pH >7.30 and respiratory rate (RR) ≤35/min (6). If undesirable hypercapnia/acidosis persisted despite RR=35/min, V_T was increased at the discretion of the treating physician. If during treatment, $PaCO_2$ was <35 mmHg and/or pH was >7.50, respiratory rate was decreased to 18–22/min and sweep gas flow was decreased to 5 L/min. Refractory hypoxemia was managed at the discretion of the attending physician (6). Circuit (hemofilter plus membrane lung) was changed every 24 hours.

The decision to interrupt $ECCO_2R$ and/or RRT was assessed daily. The potential for weaning from super-protective ventilation and $ECCO_2R$ was evaluated by switching sweep gas off and setting V_T, PEEP and RR to 6 mL/kg, 5–10 cmH_2O, and 20–30 breath/min, respectively. If the patient remained stable for at least 6 hours with a P_{PLAT} ≤25 cmH_2O and $PaCO_2$ <45 mmHg, $ECCO_2R$ was

discontinued (6). The potential for interrupting RRT was left to the attending clinician and evaluated using standard criteria (9).

"RRT-only"

A dataset for matched cohort analysis was created using data from patients with ARDS ventilated according to a conventional protective strategy (5) and enrolled in previous studies (10, 11). RRT was performed with Lynda CRRT® using a continuous veno-venous hemofiltration (CVVH) equipped with a 1.4 m^2 polisulfon hemofilter (BELLCO, Mirandola, Italy). Blood flow ranged between 200-300 ml/min with a prescribed dose of 45 ml/kg/hr (post-dilution mode 100%) and maintaining a filtration fraction lower than 25%. Patients were considered for matching if required RRT for AKI using the same criteria as for the ***"RRT-plus-ECCO$_2$R"*** group. Exclusion criteria were the same as for the ***"RRT-plus-ECCO$_2$R"*** group.

Measurements

In the ***"RRT-plus-ECCO2R"*** group, clinical data and plasma samples were collected at T0, and 24 (T1), 48 (T2) and 72 (T3) hours after reduction of V_T. In the ***"RRT-only"*** group, data analysis was performed using values collected when RRT was commenced, (T0) and 24 (T1), 48 (T2) and 72 (T3) hours after and analyzing de-novo plasma samples previously collected in the same time windows and stored at -80°.

Creatinine concentrations until RRT was interrupted, days on RRT, hospital mortality and mortality at 28 days were recorded. The reduction in creatinine with RRT was calculated as creatinine at time of initiation minus creatinine at time of interruption of RRT divided creatinine at time of initiation of RRT and expressed as % of the creatinine value at the time of initiation of RRT (9).

Potential adverse events related to ***"RRT-plus-ECCO$_2$R"*** were recorded and classified as **mechanical** (cannula problems, membrane lung failure, clots in the circuit, air in the circuit, pump malfunction, tubing rupture, catheter displacement, system leaks) and **patient-related** (vein perforation, significant bleeding [i.e. , any bleeding event that required the administration of 1 unit of packed red cells], hemodynamic instability [i.e. 80–90 mmHg increase or a 30–40 mmHg decrease in systolic blood pressure relative to the baseline value or need for inotropic drugs for at least 2 h to maintain systolic blood pressure higher than 85 mmHg or electrocardiogram evidence of ischemia or significant ventricular arrhythmias] ischemic/gangrenous bowel, pneumothorax, renal complications [i.e. occurrence after initiation of carbon dioxide removal of creatinine greater than 1.5 mg/dl], infectious complications [i.e. occurrence after initiation of carbon dioxide removal of culture proven new infection], metabolic [i.e. occurrence after initiation of carbon dioxide removal of glucose of at least 240 mg/dl or hyperbilirubinemia], thromboembolic complications [i.e. occurrence after initiation of deep venous thrombosis or pulmonary embolus], and neurologic complications [i.e. occurrence after initiation of carbon dioxide removal of cerebral infarction, or clinical seizure, or cerebral hemorrhage or cerebral edema]) (12, 13).

Assay for plasmatic concentration of interleukin-6 was carried out using a solid-phase enzyme-linked immunosorbent assay method (Diaclone, Milan, Bender Med Systems, Milan, Italy and BioSource International Inc., Camarillo, CA).

Trans-epithelial electrical resistance (TEER) was used to assess the alteration of cell permeability and polarity (14). Renal tubular epithelial cells were grown on trans wells plates with collagen-coated polycarbonate membranes (Corning Costar Corp., Cambridge, MA) and allowed to reach confluence. After 12 hours of incubation with plasma from patients of the ***"RRT-plus-ECCO$_2$R"*** group and patients from the ***"RRT-only"***, an epithelial volt-ohm meter (EVOM; World Precision Instruments, Inc., Sarasota, FL) was used to determine TEER (14). Plasma from 3 healthy

individuals was used as negative controls. All measures were normalized for the area of the membrane used in the experimental procedures (14).

Proteome Profiler Human Apoptosis Array kit (R&D Systems, Space Import-Export, Milano, MI, Italy) was used to detect expression of the following apoptosis-related proteins: Bcl-2-associated death promoter (Bad), bcl-2-associated protein X (Bax), tumor necrosis factor receptor 1 (TNF R1), Fas cluster of differentiation 95 (Fas/CD95), pro-caspase 3, cleaved caspase 3, tumor necrosis factor receptor 1 (TRAIL R1), tumor necrosis factor receptor 2 (TRAIL R2). Renal tubular epithelial cells were seeded at $1x10^6$ cells into 100 µl of DMEM for each well of a 6-well-plate (to obtain 200-600 mcg of protein from cell lysates); following 24 hours of cultivation under standard conditions, cells were starved for 6 h and then incubated over night at 37°C with plasma from patients of ***"RRT-plus-ECCO$_2$R"*** and ***"RRT-only"*** groups (plasma dilution 1:2). Images were acquired by the ChemiDoc MP system (Bio-Rad). Protein density was analyzed using a GelPro-Analyzer v 6.0 software (Media Cybernetics, Bethesda, MD, USA) (14).

All biological measurements were performed by technicians blinded to the study group.

Statistics

Values are presented as mean (standard deviation), or median (inter-quartile range). Categorical variables are shown as frequency and proportion. To reduce possible differences between patients receiving or not ***"RRT-plus-ECCO$_2$R"*** and obtain unbiased estimation of treatment effect, propensity score i.e. the probability to receiving ***"RRT-plus-ECCO$_2$R"*** conditionally on a-priori selected variables was estimated using the multivariable logistic regression analysis with ***"RRT-plus-ECCO$_2$R"*** as the dependent variable. The a priori selected variables of the logistic regression were age, Simplified Acute Physiology Score (SAPS) II, severity of AKI according to Acute Quality Dialysis Initiative (ADQI) criteria, days on mechanical ventilation, V_T, and driving

pressure. All variables were obtained at ICU admission. In a propensity score matching each patient treated with ***"RRT-plus-ECCO$_2$R"*** was matched with a patient treated with ***"RRT-only"*** with the closest propensity score selected using a Greedy algorithm. A computerized greedy matching technique was employed for this matching process whereby cases were first matched to controls that had a propensity score that was identical in all 5 digits. Those that did not match were then matched to controls on 4 digits of the propensity score. This continued down to a 1-digit match on propensity score for those that remained unmatched. The algorithm proceeds sequentially to the lowest digit match on propensity score (from 5 to 1 digits). The balance statistics was performed using the Wilcoxon-Mann-Whitney test. Values obtained at the different times were compared using analysis of variance (ANOVA) for repeated measure. If significant, the values obtained at different times were compared with those at baseline using a post hoc Dunnett test. Outcomes before and after matching were compared between treatment groups with Chi-square test or Wilcoxon-Mann-Whitney test as appropriate. All statistical tests were two-sided and p values of 0.05 or less were considered statistically significant. Statistical analyses were conducted using SAS software package (SAS Institute, Cary, NC; version 9.3) and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA 92037 USA).

Bibliography

E1. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: The berlin definition. *JAMA : the journal of the American Medical Association* 2012;307:2526-2533.

E2. Kidgo aki guidline. 2012.

E3. Tolwani A. Continuous renal-replacement therapy for acute kidney injury. *The New England Journal of Medicine* 2012.

E4. Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, Finfer S, Gardlund B, Marshall JC, Rhodes A, Artigas A, Payen D, Tenhunen J, Al-Khalidi HR, Thompson V, Janes J, Macias WL, Vangerow B, Williams MD. Drotrecogin alfa (activated) in adults with septic shock. *The New England journal of medicine* 2012;366:2055-2064.

E5. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The acute respiratory distress syndrome network. *N Engl J Med* 2000;342:1301-1308.

E6. Fanelli V, Ranieri MV, Mancebo J, Moerer O, Quintel M, Morley S, Moran I, Parrilla F, Costamagna A, Gaudiosi M, Combes A. Feasibility and safety of low-flow extracorporeal carbon dioxide removal to facilitate ultra-protective ventilation in patients with moderate acute respiratory distress sindrome. *Crit Care* 2016;20:36.

E7. Nurmohamed SA, Vervloet MG, Girbes AR, Ter Wee PM, Groeneveld AB. Continuous venovenous hemofiltration with or without predilution regional citrate anticoagulation: A prospective study. *Blood Purif* 2007;25:316-323.

E8. Aman J, Nurmohamed SA, Vervloet MG, Groeneveld AB. Metabolic effects of citrate- vs bicarbonate-based substitution fluid in continuous venovenous hemofiltration: A prospective sequential cohort study. *J Crit Care* 2010;25:120-127.

E9. Network VNARFT, Palevsky PM, Zhang JH, O'Connor TZ, Chertow GM, Crowley ST, Choudhury D, Finkel K, Kellum JA, Paganini E, Schein RM, Smith MW, Swanson KM, Thompson BT, Vijayan A, Watnick S, Star RA, Peduzzi P. Intensity of renal support in critically ill patients with acute kidney injury. *N Engl J Med* 2008;359:7-20.

E10. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, Gandini G, Herrmann P, Mascia L, Quintel M, Slutsky AS, Gattinoni L, Ranieri VM. Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2007;175:160-166.

E11. Terragni PP, Filippini C, Slutsky AS, Birocco A, Tenaglia T, Grasso S, Stripoli T, Pasero D, Urbino R, Fanelli V, Faggiano C, Mascia L, Ranieri VM. Accuracy of plateau pressure and stress index to identify injurious ventilation in patients with acute respiratory distress syndrome. *Anesthesiology* 2013;119:880-889.

E12. Del Sorbo L, Pisani L, Filippini C, Fanelli V, Fasano L, Terragni P, Dell'Amore A, Urbino R, Mascia L, Evangelista A, Antro C, D'Amato R, Sucre MJ, Simonetti U, Persico P, Nava S, Ranieri VM. Extracorporeal co2 removal in hypercapnic patients at risk of noninvasive ventilation failure: A matched cohort study with historical control. *Crit Care Med* 2014.

E13. Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, Faggiano C, Quintel M, Gattinoni L, Ranieri VM. Tidal volume lower than 6 ml/kg enhances lung protection: Role of extracorporeal carbon dioxide removal. *Anesthesiology* 2009;111:826-835.

E 14. Cantaluppi V, Assenzio B, Pasero D, Romanazzi GM, Pacitti A, Lanfranco G, Puntorieri V, Martin EL, Mascia L, Monti G, Casella G, Segoloni GP, Camussi G, Ranieri VM. Polymyxin-b hemoperfusion inactivates circulating proapoptotic factors. *Intensive Care Medicine* 2008;34:1638-1645.

Table E1. Nomogram of blood, effluent and calcium flow rates during RRT-plus- ECCO2R with citrate regional anticoagulation.

		Measured Systemic Ca_i (mmol/L)			
		0.8-0.9	**0.9-1**	**1.0-1.1**	**1.1-1.2**
Blood Flow (ml/min)	**Effluent Flow Rate (ml/hr)**	**Ca chloride (systemic infusion)**			
140	1900	5	3.7	3.2	2.5
160	2100	5.5	4.2	3.5	2.8
180	2400	6	4.7	4	3.3
200	2700	6.7	5.3	4.5	3.7
220	3000	7.3	6	5	4
240	3200	7.7	6.5	5.3	4.2
260	3400	8.5	7.2	5.8	4.5
280	3500	9.6	7.8	6.2	4.8
300	3600	10.7	8.3	6.6	5.1
350	3700	11.8	8.8	7	5.4

Definition of abbreviations. Ca: calcium; Ca_i: calcium ionized

Table E2. Baseline Characteristics of Patients in RRT-plus-ECCO2R and RRT-only groups.

Variables	**RRT-*plus*-$ECCO_2R$ (n=14)**	**RRT-only (n=40)**	**p**
Gender – n (%)			
Male	8 (57)	32 (80)	0.09000
ARDS risk factors – n (%)			0.2841
Abdominal septic shock	4(29)	11(28)	
Pneumonia	7(50)	18(45)	
Acute pancreatitis	1(7)	2(5)	
Thoracic trauma	1(7)	5(12)	
Polytrauma	1(7)	4(10)	
APACHE II	25.8(6)	25(7.6)	0.7669
AKI etiology – n (%)			0.1064
Sepsis	11(79)	23(58)	
Nephrotoxic drugs	1(7)	0(0)	
Ischemia	2(14)	12(30)	
Multifactorial causes	0(0)	5(12)	

Definition of abbreviations. ARDS: Acute Respiratory Distress Syndrome; APACHE II: Acute Physiology and Chronic Health Evaluation II; AKI: acute Kidney Injury.

Table E3. Time course of ventilator variable and gas exchange in the RRT-plus-ECCO$_2$R and RRT-only groups.

	T0		T1		T2		T3	
	RRT-*plus*-ECCO$_2$R	**RRT-only**	**RRT-*plus*-ECCO$_2$R**	**RRT-only**	**RRT-*plus*-ECCO$_2$R**	**RRT-only**	**RRT-*plus*-ECCO$_2$R**	**RRT-only**
VT	7.04(0.5)	7.06(0.6)	4.86(0.4)	6.93(0.5)	4.83(0.4)	7.15(0.7)	4.84(0.4)	7.2(0.8)
RR (b/min)	22 (7)	23 (6)	26 (8)	22 (5)	26 (8)	22 (7)	27 (9)	23 (6)
Pplat (cmH_2O)	28.1 (1.6)	27.7 (1.4)	25.1 (2.4)*	27.5 (1.1)	24.8 (1.8)*	27.4 (1)	24.6 (2)*	27.7 (0.7)
PEEP (cmH_2O)	9.4 (3.7)	8.5 (2)	10.8 (3.7)	8.6 (1.5)	10.6 (2.6)	9.5 (2)	11.3 (3.8)*	9.6 (2)
Delta P (cmH_2O)	19(2.2)	19(2.3)	15(2.6) *	19(1.3)	14(2.5) *	18(2)	14(2.1) *	18(2)
$PaCO_2$ (mmHg)	46(7.5)	42(6.9)	48(7.1)	44(9)	48(7)	42(6.5)	47(7.3)	41(5.7)
pH	7.35(0.1)	7.37(0.1)	7.36(0.1)	7.37(0.1)	7.38(0.05)	7.37(0.05)	7.39(0.05)	7.37(0.06)
HCO_3^- (mEq/L)	26(5)	23(4.6)	28(6)	25.2(3.1)	29.7(6)	24.3(3.2)	29.6(5.5)	25(2.4)
FiO_2	0.5(0.2)	0.5(0.2)	0.5(0.2)	0.4(0.2)	0.5(0.2)	0.5(0.1)	0.6(0.2)	0.4(0.1)
PaO_2 (mmHg)	110(37)	106(25)	100(27)	102(21)	95(25)	95(12)	102(10)	95(16)

Definition of abbreviations. VT: Tidal Volume; RR: Respiratory Rate; Pplat: Plateau Pressure; PEEP: Positive End Expiratory Pressure; Delta P: Driving Pressure (Pplat-PEEP); $PaCO_2$: Arterial Pressure of Carbon Dioxide; FiO_2: Inspired Fraction of Oxygen; PaO_2: Arterial Pressure of Oxygen.

*p<.05 vs T0.

Table E4. Time course of operational characteristics of RRT in the two study groups.

Variables	T0		T1		T2		T3	
	RRT-*plus*-ECCO$_2$R n=13	**RRT-only** n=13	**RRT-*plus*-ECCO$_2$R** n=13	**RRT-only** n=13	**RRT-*plus*-ECCO$_2$R** n=13	**RRTonly** n=13	**RRT-*plus*-ECCO$_2$R** n=13	**RRT-only** n=13
Effluent flow rate (ml/kg/h)								
Heparin	38(4.7)	39.6(8)	35(4.3)	41.6(8)	34(1)	38.7(7.5)	37(4.2)	38.9(8)
Citrate	38(4.6)	-	38(12.6)	-	37(6)	-	38(4)	-
Blood Flow (ml/min)								
Heparin	268(78)	200(8)	310(35)	220(10)	332(26)	200(0)	325(30)	180(30)
Citrate	283(23)	-	266(47)	-	307(19)	-	310(26)	-
Type of Anticoagulation								
Heparin								
Dose (UI/kg/h)	14(5)	3(1)	15(3)	3(0.6)	12(4)	4(0.2)	12(2)	3.5(0.4)
Citrate								
Ca infused (mmol/h)	4.5(2)	-	6(1.4)	-	6.2(1.6)	-	7(1.2)	-
Ca blood (mEq/L)	1(0.08)	-	1.2(0.01)	-	1(0.02)	-	0.98(0.07)	-
Fluid Removal (ml/h)								
Heparin	115(9)	122(82)	106(14)	126(71)	101(10)	134(72)	97(9)	154(64)
Citrate	115(7)	-	110(14)	-	108(18)	-	101(23)	-
Arterial Pressure (mmHg) *Heparin*	-33(21)	-31(24)	-59(16)	-38(12)	-67(14)	-30(13)	-73(15)	-34(15)
Citrate	-39(14)	-	-56(19)	-	-75(15)	-	-72(11)	-
Venous Pressure (mmHg)								
Heparin	55(30)	46(32)	80(10)	34(19)	81(16)	38(18)	88(10)	29(10)
Citrate	51(17)	-	70(22)	-	76(15)	-	82(8)	-
Transmembrane Pressure (mmHg)								
Heparin	18(13)	20(15)	54(26)	22(19)	39(33)	25(13)	40(33)	18(6)
Citrate	14(3)	-	47(12)	-	32(12)	-	37(18)	-

Definition of abbreviations. RRT: Renal Replacement Therapy.