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

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Running Title: $ECCO_2R$ 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; AC: Patient recruitment, data collection and manuscript revision; PC: Data collection and analysis, 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; 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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To the editor:

Introduction

Attenuation of inflammatory and apoptotic response in patients with acute respiratory distress syndrome (ARDS) have been associated to the reduction in end-organs failure and the improvement in outcome observed with conventional protective ventilation (1). Recent data show that further reduction of tidal volume (V_T) improves outcome but extracorporeal CO_2 removal ($ECCO_2R$) is needed to manage respiratory acidosis (2).

Mechanical ventilation is an independent risk factor of mortality in patients with acute kidney injury (AKI) (3). Increased plasma concentration of inflammatory mediators and apoptosis of renal tubular cells are associated with AKI (4).

Recent studies propose to incorporate $ECCO_2R$ in conventional renal replacement therapy (RRT) circuit to simultaneously support lung and kidney functions (5, 6). However, data comparing RRT+$ECCO_2R$ (RRT+) with ultra-protective ventilation and RRT only with conventional ventilation are not available. This study set up to examine the hypothesis that adding RRT+ allows ultra-protective ventilation that preserves renal function through attenuation of inflammation and apoptosis.

Methods

Mechanically ventilated ARDS patients on RRT for AKI were enrolled in the period December 2015-March 2017 (**ClinicalTrials.gov Identifier:** NCT02595619). Review boards approved the protocol.

RRT+

RRT was performed with continuous veno-venous hemodiafiltration. A polypropylene membrane lung was inserted in series before the hemofilter (Diapact CRRT®; B.Braun). Anticoagulation was ensured by continuous infusion of heparin (2). In case of contraindication to heparin, a calcium-free citrate replacement fluid provided anticoagulation. RRT was commenced at a blood flow of 300 ml/min. Sweep gas was set at 0 L/min (time 0, T0). V_T was hence reduced from 6 to a minimum value of 4 ml/kg while increasing PEEP to target a P_{PLAT} of 25 cmH_2O (2). Once the lowest value of V_T was reached, sweep gas was switched on (10 L/min) to obtain $PaCO_2$ values similar to baseline (±20%) with a pH >7.30 and respiratory rate ≤35/min (2).

RRT

A dataset for matched cohort analysis was created using data from patients on RRT enrolled in previous studies (7, 8) and ventilated with conventional V_T. RRT was performed using continuous veno-venous hemofiltration (Lynda CRRT®; BELLCO). Anticoagulation was ensured by continuous infusion of heparin.

In ***RRT+***, clinical data and plasma samples were collected at T0, and every 24 hours at 8 a.m. (T1, T2, T3) after reduction of V_T and at same time points in ***RRT***. Creatinine concentrations until discontinuation of RRT, days on RRT, hospital and mortality at 28 days

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were recorded. Side-effects were (a) defined as in a previous study (2) and (b) prospectively recorded.

Plasmatic concentration of interleukin-6 (IL6) and expression of proteins related to apoptosis [B-cell lymphoma 2 -associated death promoter (Bad) and X protein (Bax); 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)] were measured (4). Alteration of renal tubular epithelial cell permeability and polarity was assessed measuring trans-epithelial electrical resistance (TEER). Plasma from heathy individuals was used as negative control (4).

A regression logistic model was used to produce a Propensity Score for patient treated with RRT+, using the following covariates: age, SAPS II score, KDIGO class, days of mechanical ventilation before RRT, tidal volume, driving pressure. Each patient treated with ***RRT***+ was hence matched with a patient treated with ***RRT*** using the Greedy algorithm. This method uses the nearest available pair matching method based on Propensity Score. The patient treated with ***RRT***+ (cases) are ordered and sequentially matched to the nearest unmatched patient treated with ***RRT*** (control). If more than one unmatched control matches to a case, the control is randomly selected. The algorithm proceeds sequentially to the lowest digit match on propensity score, with a caliper defined on five digits of the Propensity Score. Goodness of matched pairs is defined as those with the least absolute difference in matched propensity score (9).

Values obtained at the different times were compared using paired t-test or analysis of variance for repeated measure with 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. Analysis was performed using SAS 9.4 (SAS Institute Inc.) and the SAS %MACRO GREEDMTCH.

Results

Eighty-five patients were admitted and 14 were treated with ***RRT***+ being eligible for matching. Of the 143 patients enrolled in previous studies (7, 8), 40 patients were treated with ***RRT*** and were eligible for matching. Propensity score identified for comparisons 13 patients for ***RRT***+ to be compared with ***RRT*** (**Table 1**).

Creatinine decreased from 2.82±1.04 to 1.49±0.76 ($p<0.0001$) and from 3.49±1.15 to 2.72±1.28 mg/dl ($p=0.0128$) in ***RRT***+ and ***RRT***, respectively. Creatinine reduction was larger and days on renal replacement therapy were smaller in ***RRT***+ than ***RRT*** (49±20 vs. 26±22% and 7±3 vs. 10±4 days, respectively $p<0.05$). In ***RRT***+, IL6 significantly decreased over time ($p<0.05$) and TEER returned to values of healthy controls (**Figure 1A**). Expression of all studied apoptosis related proteins are shown in **Figure 1B**. Compared with baseline, expression of Bad, Fas and TRAIL R2 significantly decreased in ***RRT***+ while remained stable in RRT. Of note, expression of cleaved caspase 3 and TNF R1 significantly increased only in ***RRT*** **(Figure 1B)**.

Mean arterial pressure, effluent flow rate and the amount of fluid removal did not differ between groups. Blood flow was higher in ***RRT***+ than in ***RRT*** (276±53 vs. 200±8, 286±46 vs. 220±10, 318±25 vs. 220±10, 317±28 vs 180±30 in ***RRT***+ vs ***RRT*** at T0, T1, T2 and T3, respectively; $p<0.05$). In ***RRT***+, six patients received heparin and seven citrate. Heparin dose was higher during ***RRT***+ than ***RRT*** (14 vs 3 UI/kg/h, respectively; $p<0.05$). In the seven patients who received local citrate anticoagulation, infusion of Ca chloride ranged between 4.5±2 and 7±1.2 mmol/h and blood concentration of Ca ionized ranged between 0.98±0.07 and 1.2±0.01 mEq/L.

In ***RRT***+, activation of sweep gas (8.1±0.5, 8.1±0.6 and 8 L/min at T1, T2 and T3, respectively) allowed to decrease V_T and driving pressure from 7.04±0.5 and 19.2±2.2 at

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baseline to 4.84±0.4 and 14.1±2.1 at T3, respectively ($p<0.05$). V_T and driving pressure did not change in ***RRT*** (7.06±0.6 and 19.1±2.2 at T0 vs 7.2±0.8 and 18.1±1.8 at T3, respectively; $p=0.17$). In ***RRT***+, PEEP was increased being significantly higher at T3 than at T0 (11.3±3.8 vs 9.4±3.7; $p<0.05$). $PaCO_2$, pH, PaO_2/FiO_2 ratio remained stable throughout the study period in both groups.

No adverse events were reported either with citrate and heparin anticoagulation

Discussion

We show that RRT and $ECCO_2R$ may be performed simultaneously in ARDS patients that develop AKI allowing the use of ultra-protective ventilation while supporting renal function. Recovery of renal function was more pronounced and concentration of inflammatory and pro-apoptotic mediators was lower when ultra-protective ventilation was allowed by ***RRT+*** than when conventional ventilator settings were used during ***RRT***. These results support the hypothesis that reduction of mechanical stress on the lungs mitigates end-organs failure and that the attenuation of pro-inflammatory and pro-apoptotic tone is the putative underlying mechanism (1).

Contrary to previous studies (2), no adverse events either related to $ECCO_2R$, anticoagulation (heparin vs. citrate) and/or serum concentration of calcium were reported with ***RRT+.*** 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 (10).

Risk of atelectasis and hypoxemia during $ECCO_2R$ was prevented by a 20% increase in PEEP that maintained PaO_2/FiO_2 unchanged throughout the study period. Although these data may provide the rationale for future randomized clinical trials, the following major limitations need to be acknowledged. First, RRT circuits and anticoagulation strategies differed between ***RRT+*** and ***RRT***. Second, although differences between groups were minimized by the adopted matching method, small and not significant differences in creatinine values (2.82±1.04 vs 3.49±1.15 mg/dl in RRT+ and RRT, respectively; p=0.14) and KDIGO class II (number of patients 2(15%) vs 1(8%) in RRT+ and RRT, respectively; p=0.99) and class III (number of patients 11(85%) vs 12(92%) in RRT+ and RRT, respectively; p=0.99) were still observed. Third, the non-randomized design and unmeasured

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residual confounders may systematically overestimate the magnitude of the beneficial effects and of the safety profile of ***RRT+***.

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Table 1. Baseline characteristics of patients before and after matching.

	RRT+ **(n=14)**	***RRT*** **(n=40)**	***RRT+*** **(n=13)**	***RRT*** **(n=13)**
	Before Matching		***After Matching***	
Age (yrs)	57(22)	63(13)	60(20)	60(13)
SAPS II	54(12)	47(10)	53(12)	52(16)
KDIGO				
I n (%)	0(0)	0(0)	0(0)	0(0)
II n (%)	2(14)	8(20)	2(15)	1(8)
III n (%)	12(86)	32(80)	11(85)	12(92)
Days of MV before RRT	6.8(4.7)	11(3.5)	7.8(3.5)	7.4(4.4)
V_T T0 (ml/kg/PBW)	7.2(0.6)	7.0(0.6)	7.03(0.46)	7.06(0.58)
Driving Pressure (cmH_2O)	19.3(3.9)	18.4(2.6)	19.2(2.2)	19.2(2.2)

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

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Figure Legends

Figure 1. Time course of plasmatic concentration of interleukin 6 (IL6) in patients treated with ***RRT***+ and ***RRT***. §p<.05 ***RRT*** T3 vs. T0; *p<.05 ***RRT***+ vs ***RRT***. (**panel A left**). Trans-epithelial resistance (TEER) of renal tubular cells after incubation with plasma from patients of ***RRT***+ and ***RRT*** groups. § p<.05 T3 vs T0 (**panel A right**). 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, tumor necrosis factor receptor 1 (TNFR1), Fas cluster of differentiation 95 (Fas/CD95), tumor necrosis factor receptor 1 (TRAIL R1), tumor necrosis factor receptor 2 (TRAIL R2) after stimulation with plasma obtained from patients treated with ***RRT***+ and ***RRT*** at T0 and T3. °p<.05 ***RRT*** at T3 vs T0. *p<.05 ***RRT***+ at T3 vs T0 (**panel B**).

Figure 1