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TITOLO TESI

**“Acute Kidney Injury and Sepsis in Intensive Care Unit:
Clinical, Biochemical, Instrumental Evaluation
and the role of Emerging treatments”**

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Abbreviations

AAA	Abdominal Aortic Aneurysm
AKI	Acute Kidney Injury
AOPP	Advanced oxidation protein products
BIVA	Bio Impedence Vector Analysis
BNP	Brain Natriuretic Peptide
CPFA	Coupled Plasma Filtration Adsorption
CHF	Congestive Heart Failure
CRS	Cardio-Renal Syndrome
CVP	Central Venous Pressure
CVVH	Continuous Veno-Venous Hemofiltration
GFR	Glomerular Filtration Rate
HVHF	High-Volume Hemofiltration
ICU	Intensive Care Unit
NGAL	Neutrophil Gelatinase-Associated Lipocalin
OS	Oxidative Stress
pHVHF	pulse High-Volume Hemofiltration
ROS	Reactive Oxygen Species
SIDa	apparent Strong Ion Difference
SIDe	effective Strong Ion Difference
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
SVV	Stroke Volume Variations

SUMMARY

Sepsis is a primary cause of morbidity and mortality in intensive care unit (ICU) and critically ill patients. Acute Kidney Injury (AKI), a frequent complication in critically ill septic patients, occurs in 35-65% of ICU admissions and is an independent risk factor for increased mortality.

Sepsis is also a contributing factor in more than 20% of cases of AKI in ICU patients, with cases severe enough to require renal replacement therapy.

The higher mortality of these patients required an accurate and early diagnosis of AKI-Septic status as soon as an accurate prevention of kidney failure main complications: fluid overload and acidosis.

At the same time we need new extracorporeal treatments not only by offering renal replacement with AKI-related treatments, but also by providing multi-organ support therapies for other organs involved in septic shock.

We conduct a series of studies to investigate the role of new technologies for clinical, instrumental and biochemical evaluation in Septic and Non septic-AKI patients; we also analyzed which extracorporeal treatments can allow improve patients clinical patterns and outcome.

Biochemical Markers of Acute Kidney Injury

We investigate the possible correlation between serum biochemical markers of organ damage, such as Neutrophil Gelatinase-Associated Lipocalin (NGAL), Advanced Oxidation Protein Products (AOPP) and Brain Natriuretic Peptide (BNP) in ICU-AKI patients with or without sepsis.

Ninety-eight consecutive adult patients admitted to ICU, divided in two groups depending on the presence of sepsis were enrolled.

In Ninety-eight adult patients admitted to ICU the levels of NGAL, BNP and AOPP were significantly higher among septic patients compared with non septic subjects ($p < 0.001$).

Among septic patients, subjects who developed AKI showed significant higher levels of NGAL and AOPP ($p = 0.0425$), and BNP ($p = 0.0327$).

Fluid status management: BIVA and SVV

Bioelectrical Impedance Vector Analysis (BIVA) could be useful the assessment of tissue hydration status in critically patients.

A cross-sectional study of 34 patients admitted to the Intensive Care Unit on mechanical ventilation was performed: patients were classified by resistance(Rz) / height(H) ratio (Ω/m): $>200\Omega/m = \text{dry}$, $\leq 200\Omega/m = \text{wet}$.

Patients categorized by Rz/H as wet showed a weak but clinically congruent association with a higher CVP (13.0 vs 9.9 mmHg, $p=0.065$) and higher BNP (882 vs 352 pg/ml, $p=0.083$).

Stroke Volume Variations (SVV) is a dynamic cardiac preload parameter, and its wide variations are described in the literature as a predictor of volume responsiveness in several populations of mechanically ventilated patients.

We conducted a pilot study in patients who undergo surgical interventions of abdominal aortic aneurysm (AAA) surgery to assess if with wide variations of SVV before and after clamping of the aorta are correlated with an higher risk of AKI development than those with lesser variations.

As compared to patients without AKI, AKI patients had a significantly larger SVV after aortic clamping (13.25 vs. 24.5, $p=0.01$). The increase in SVV at aortic declamping time, as compared to SSV at clamping time, was also significantly higher in AKI patients (-3.75 vs. 12.5, $p=0.04$).

Management and Detection Of Acidosis

Acid-base disorders are common in the ICU and are indications for Renal Replacement Therapies (RRT). The Stewart Approach may be superior for acid-base analysis in the critically ill.

We enrolled 19 consecutive adult patients on CVVH and mechanical ventilation.

We calculated $[HCO_3^-]$ and SBE with the Henderson- Hasselbach and Siggaard-Andersen equations. Physicochemical analysis was performed using the Stewart equations modified by Figge et al. The apparent strong ion difference (SIDa) and the effective strong ion difference (SIDE) were then calculated.

The prevalence of acidosis after CVVH, as assessed by pH vs. SIDE, was [36.8% vs. 94.7% ($p<0.001$)] at 6h, 21.1% vs. 73.7% ($p<0.05$) at 12h, and [21.1% vs. 98.6% ($p<0.001$)] at 24h.

The prevalence of acidosis after CVVH, as assessed by SBE vs. SIDE, was

[57.9% vs.94.7%(p<0.05)] at 6h,[63.2% vs.73.7% (p=NS)]at 12 h,and [63.2% vs.98.6%(p<0.05)] at 24h.

News Extracorporeal Treatments for AKI and Sepsis

High Volume Hemofiltration (HVHF) and Coupled Plasma Filtration Adsorption (CPFA) have shown potential improvements in septic animals, but actually there are no studies that compare these two types of treatments in human.

Our aim was to compare the hemodynamic effects of HVHF and CPFA in septic shock patients with Acute Kidney Injury (AKI) undergoing Continuous Renal Replacement therapies(CRRT). We performed a cross-over study enrolling patients with septic shock and AKI who were receiving CRRT. The primary endpoints were changes in mean arterial pressure, vasopressors requirement (expressed as *Vasopressor Score*, VS) and changes in noradrenaline dose after pHVHF and CPFA. There was a trend for reduction in VS with HVHF and CPFA (HVHF p= 0.13, CPFA p< 0.05) There was not a significant difference between the two treatments in terms of percentage change in VS score (p=0.22).

New Technologies, New Biochemical parameters and new treatments can improve early diagnosis, monitoring and treatment in AKI septic and Non-Septic patients.

SOMMARIO

La Sepsis rappresenta una delle principali cause di morbilità e mortalità nelle Unità di Terapia Intensiva (ICU): l'insufficienza renale acuta (Acute Kidney Injury AKI) è una frequente complicanza nel 35-65 % dei pazienti in ICU è qualora presente, comporta un incremento della mortalità, ancora più evidente qualora vi sia la necessità di instaurare una Terapia di Supporto della Funzione Renale (Renal Replacement Therapy RRT).

La Sepsis rappresenta il fattore eziopatogenetico principale in più del 20% dei casi di AKI: la sua repentina presentazione clinica e la elevata mortalità richiedono una diagnosi precoce e una altrettanto rapida individuazione di AKI nel paziente con sepsi, nonché una adeguata valutazione delle principali complicazioni della insufficienza renale acuta: il sovraccarico idrico (Volume Overload) e l'acidosi.

Allo stesso tempo si rende indispensabile la attuazione di nuovi trattamenti depurativi, atti non solo a ripristinare la funzionalità renale, ma anche a fornire una funzione di supporto ad altri organi e apparati coinvolti nella Sepsis.

Abbiamo condotto una serie di studi clinici con lo scopo di investigare le nuove tecnologie per la diagnosi precoce della insufficienza renale acuta nei pazienti con e senza sepsi, nonché la valutazione di nuove metodiche nella identificazione e nel management del sovraccarico idrico e della acidosi; infine abbiamo analizzato il potenziale ruolo dei trattamenti emergenti nei pazienti con AKI e Sepsis.

Markers Precoci di Insufficienza Renale Acuta

Abbiamo analizzato la correlazione tra indicatori precoci di danno d'organo come la Neutrophil Gelatinase-Associated Lipocalin (NGAL), i Prodotti di Avanzata Ossidazione delle Proteine (Advanced Oxidation Protein Products AOPP) e il Peptide Natriuretico Atriale (Brain Natriuretic Peptide BNP) in 98 pazienti in ICU con AKI, studiando il diverso comportamento di questi marcatori in pazienti con o senza sepsi.

I livelli di NGAL, BNP e AOPP erano significativamente più elevati nei pazienti con sepsi rispetto ai pazienti senza sepsi ($p < 0.001$); inoltre i pazienti con sepsi che sviluppavano AKI

presentavano valori ancora più elevati di NGAL, AOPP (p=0.0425 e BNP (p=0.0327) rispetto ai pazienti con sepsi senza AKI.

Valutazione del “Fluid Status”: BIVA and SVV

La Bioimpedenza (Bioelectrical Impedance Vector Analysis BIVA), metodica da tempo utilizzata nel paziente cronico, potrebbe essere utile nella valutazione dello stato di idratazione nel paziente critico: in tal senso ne abbiamo valutato il ruolo in 34 pazienti in ICU.

I pazienti sono stati classificati utilizzando il rapporto Resistenza (Rz) / Altezza (H) (Ω/m): se superiore a $200\Omega/m$ = ipoidratati, se inferiore o uguale a $\leq 200\Omega/m$ = Iperidratati.

I pazienti classificati come iperidratati mostravano una correlazione positiva con elevati valori di Pressione Venosa Centrale (Central Venous Pressure CVP) (13.0 vs 9.9 mmHg, p=0.065) ed elevati valori di BNP (882 vs 352 pg/ml, p=0.083).

Lo *Stroke Volume Variations (SVV)* è un indice indiretto di “preload” ed è descritto in letteratura come un indice predittivo “dinamico” dello stato di idratazione.

Abbiamo condotto uno studio pilota in pazienti sottoposti ad intervento di Aneurisma dell’Aorta Addominale (Abdominal Aortic Aneurysm AAA) analizzando se le variazioni dello SVV prima e dopo il clamp aortico sovra renale potessero essere correlate ad AKI.

I pazienti che presentavano AKI dopo l’intervento avevano valori di SVV più elevati dopo il clampaggio dell’ aorta (13.25 vs. 24.5, p=0.01) e un maggiore incremento dei valori di SVV dopo il declampaggio (-3.75 vs. 12.5, p=0.04).

Valutazione e Management dell’Acidosi

I disturbi dell’equilibrio acido-base sono molto diffusi in ICU e rappresentano una comune indicazione alla Terapia Renale Sostitutiva (RRT)

In diversi studi clinici l’approccio di Stewart sembra essere superiore all’approccio “tradizionale” nel paziente critico.

In 19 pazienti con AKI e trattati con Emofiltrazione Venovenosa Continua (Continuous Venovenous Hemofiltration CVVH) abbiamo valutato la concentrazione di Bicarbonato [HCO_3^-] e lo Standard Base Excess [SBE] con le equazioni di Henderson- Hasselbach e Siggaard-Andersen e le abbiamo paragonate con le equazioni di Stewart, valutandone le differenze.

Per l'analisi fisico-chimica di Stewart abbiamo calcolato la "Apparente Differenza tra ioni forti" (Apparent Strong Ion SIDa e la "Effettiva Differenza Tra gli Ioni Forti" (Effective Strong Ion Difference SDe).

La prevalenza di acidosi durante la CVVH, valutata come pH vs. SDe era [36.8% vs. 94.7% (p<0.001)] nelle prime 6 ore, 21.1% vs. 73.7% (p<0.05)] a 12h, e [21.1% vs. 98.6% (p<0.001)] a 24h. La prevalenza di acidosi durante la CVVH intesa come SBE vs.SDe era 57.9%vs.94.7%(p<0.05)] a 6h, [63.2% vs.73.7% (p=NS)] e 12 h, e [63.2% vs.98.6%(p<0.05)] a 24h.

Tale approccio appare più sensibile rispetto al tradizionale nella diagnosi di acidosi nel paziente critico in CVVH.

Trattamenti Extracorporei Emergenti per AKI e Sepsis:

La Emofiltrazione ad alti volume di Reinfusione (High Volume Hemofiltration HVHF) e la Coupled Plasma Filtration Adsorption (CPFA) hanno mostrato incoraggianti risultati in modelli animali e nei primi studi sperimentali sull'uomo.

Scopo del nostro studio è stato comparare questi due trattamenti nei pazienti con sepsi e AKI.

Dai dati in nostro possesso entrambi i trattamenti permettono un miglioramento dei parametri emodinamici e del fabbisogno di vasopressori espresso come *Vasopressor Score* VS, (HVHF p= 0.013, CPFA p< 0.05) senza sostanziali differenze tra i due.

I nuovi indici biochimici, le nuove metodiche strumentali, invasive e non invasive permettono una diagnosi precoce e ottimizzano la gestione del paziente con AKI e Sepsis. I trattamenti emergenti sembrano rappresentare una valida alternativa all'attuale standard terapeutico e potrebbero in futuro migliorarne la prognosi.

INTRODUCTION

Sepsis

Sepsis is a primary cause of morbidity and mortality in Intensive Care Unit (ICU) and critically ill patients [1].

Sepsis occurs in 1-2% of all hospitalizations and accounts for as much as 25% of ICU bed utilization. It is a major cause of death in ICU worldwide, with mortality rates that range from 20% for sepsis to 40% for severe sepsis to 60% for septic shock [1-2].

Septic shock is also a strong predictor of short- and long-term mortality. Case-fatality rates are similar for culture-positive and culture-negative severe sepsis [3].

It is known that approximately 20-35% of patients with severe sepsis and 40-60% of patients with septic shock die within 30 days, and others die within the ensuing 6 months. Late deaths often results from poorly controlled infections, immuno-suppression, complications of intensive care, failure of multiple organs, or the patient's underlying disease [1].

The pathogenesis of these syndromes is becoming increasingly understood and it's hoped that this will result in improved outcome [4].

Sepsis is an extremely complex process that involves the activation of inflammatory, coagulation and complement cascades as well as production of pro- and anti-inflammatory cytokines [5].

It is characterized by a systemic response that often varies depending on the type of pathogen, patient age and co-morbidities, as well as genetic factors. The non-linear complexity is largely determined by the interplay of cells involved in the systemic response. These include: monocytes, lymphocytes, neutrophils, dendritic cells, platelets and endothelial cells [5].

Attempts at inactivating single mediators or pathways have largely failed, and efforts currently focus on targeting the amplified response to infection by developing broader based therapies [6].

In the last years extracorporeal treatments have found new applications, not only by offering renal replacement with AKI-related treatments, but also by providing multi-organ support therapies for other organs involved in septic shock [7].

The modern concept of sepsis is that the host's immune response to the infection causes most of the symptoms of sepsis, resulting in hemodynamic consequences and damage to organs. This

host response has been termed Systemic Inflammatory Response Syndrome (SIRS) and is characterized by hemodynamic compromise and resultant metabolic derangement [8].

In 1991, the American Collage of Chest Physicians and the American Society of Critical Care Medicine published definitions for the SIRS and sepsis, with the aim of clarifying the diagnosis and treatment of these conditions and to aid interpretation of research in this field [9].

There are different levels of sepsis [Table1].

Table1: Definitions for SIRS and Sepsis

<p>SIRS</p>	<p>Defined by the presence of two or more of the findings</p>	<ul style="list-style-type: none"> * Body Temperature <36°C or >38°C * Heart Rate >90 beats per minute * Respiratory Rate >20 breaths per minute or a PaCO₂ <32mm Hg * WBC <4,000 cells/mm³ or >12,000 cells/mm³
<p>Sepsis</p>	<p>Defined as SIRS in response to a confirmed infectious process</p>	<p>Infection can be suspected or proved by</p> <ul style="list-style-type: none"> * Culture * Stain * PCR <p>or a clinical syndrome pathognomonic for infection</p>
<p>Severe Sepsis</p>	<p>Defined as sepsis with organ dysfunction, hypoperfusion, or hypotension</p>	
<p>Septic Shock</p>	<p>Defined as sepsis with refractory arterial hypotension or hypoperfusion may be either end-organ dysfunction or serum lactate greater than 4 mmol/dL</p>	<p>End-organ dysfunction include [5]</p> <ul style="list-style-type: none"> * Lungs * Brain * Liver * Kidney * Heart

Consensus definitions, however, continue to evolve, with the latest expanding the list of signs and symptoms of sepsis to reflect clinical bedside experience.

Prognosis stratification systems such as Sequential Organ Failure Assessment (SOFA) score indicate that factoring in various physiologic variables can yield estimates of the risk of dying of severe sepsis.

The SOFA score is used to track a patient's status during the stay in an ICU and it is one of several ICU scoring systems[10].

In the ICU, gram-negative bacteria are implicated in 50 to 60% of sepsis with gram-positive bacteria accounting for a further 35 to 40% of cases. The remainder of causes are due to the less common causes of fungi, viruses and protozoa.

AKI

Acute Kidney Injury (AKI) is a rapid loss of kidney function. AKI is common among hospitalized patients. It affects some 3-7% of patients admitted to the hospital and approximately 25-30% of patients in the ICU [11].

The aetiology of AKI in critically ill patients is often multifactorial. However, sepsis has consistently been found to be a leading contributing factor to AKI in critical illness. Discriminating between AKI of septic and non septic origin may have clinical relevance. Evolving data suggest that septic AKI may be characterized by a distinct pathophysiology.

Septic AKI occurs between 15% and 20% of all ICU admissions and its mortality ranges from 20% to 60%. The incidence and mortality of septic AKI has remained high throughout the last 10 years, whereas our understanding of septic AKI pathogenesis has remained limited [11].

The diagnosis of AKI is generally made using two markers: urine output and plasma creatinine.

The use of urine output and creatinine has dominated the clinical scenario for many years. They represent important tools since they are the foundations of criteria for diagnosing AKI in RIFLE classification system [11-12].

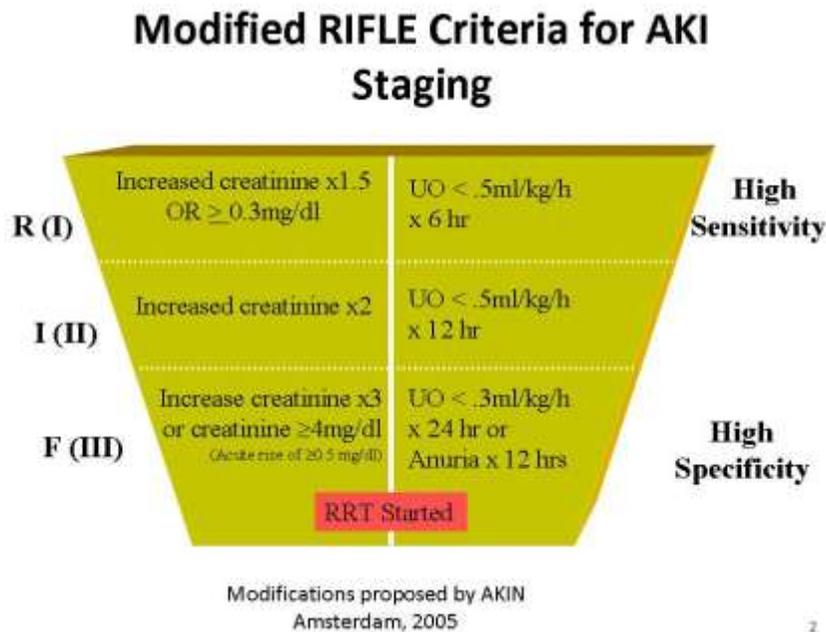
Actually, in response to the need for a common meaning for AKI, because AKI has been, over the last few decades the focus of extensive clinical research efforts, the Acute Dialysis Quality Initiative Group, a panel of international experts in nephrology and clinical care medicine, developed and published a set of consensus criteria for a uniform definition and classification of AKI. These RIFLE criteria classify renal dysfunction according to the degree of impairment present: there are three grades of severity (Risk, Injury, and Failure), and two outcome classes (Loss of kidney function, and End-stage kidney disease) [Figure1].

The RIFLE classification has been evaluated and validated in numerous clinical studies enrolling critically ill patients namely post-operative patients and burned patients, and found to be a valid tool for the precocity of the diagnosis and staging of AKI, having predictive ability for mortality [13].

The diagnosis and etiological classification of AKI, at present, largely depend on the detection of changes in conventional endogenous surrogate markers of kidney function, especially serum levels of creatinine and urea. These tests are familiar to clinicians and have long been used at the bedside. Regrettably, however, these markers are not ideal, each has limitations, none reflect real-

time dynamic changes in Glomerular Filtration Rate (GFR), and none reflect genuine kidney injury.

Figure1: RIFLE Classification. RIFLE class is determined according to the worst degree of either Glomerular Filtration Rate criteria (according to the creatinine values) or urine output criteria.



Sepsis is also a contributing factor in more than 20% of cases of acute kidney injury (AKI) in ICU patients, with cases severe enough to require renal replacement therapy. AKI occurs in 35-65% of ICU admissions and most studies show a threefold to fivefold increase in the risk of death among patients with AKI compared to patients without AKI. Acute Renal Failure (ARF), a frequent complication in critically ill septic patients, is an independent risk factor for increased mortality, particularly when patients require renal replacement therapy (RRT) [14].

The effects of this large complex required an accurate and early diagnosis of AKI-Septic status as soon as an accurate prevention of kidney failure complications: fluid overload and acidosis.

In the last years extracorporeal treatments have found new applications, not only by offering renal replacement with AKI-related treatments, but also by providing multi-organ support therapies for other organs involved in septic shock [15].

Cardio-Renal Syndrome

A large proportion of patients admitted to hospital, especially in the critical care setting, have various degree of heart and kidney dysfunction. Primary disorders of one of these two organs often result in secondary dysfunction or injury to the other.

The general definition of Cardio-Renal Syndrome (CRS) is a disorder of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other, and it has been expanded into five subtypes reflecting the primacy of organ dysfunction and the time-frame of the syndrome [16].

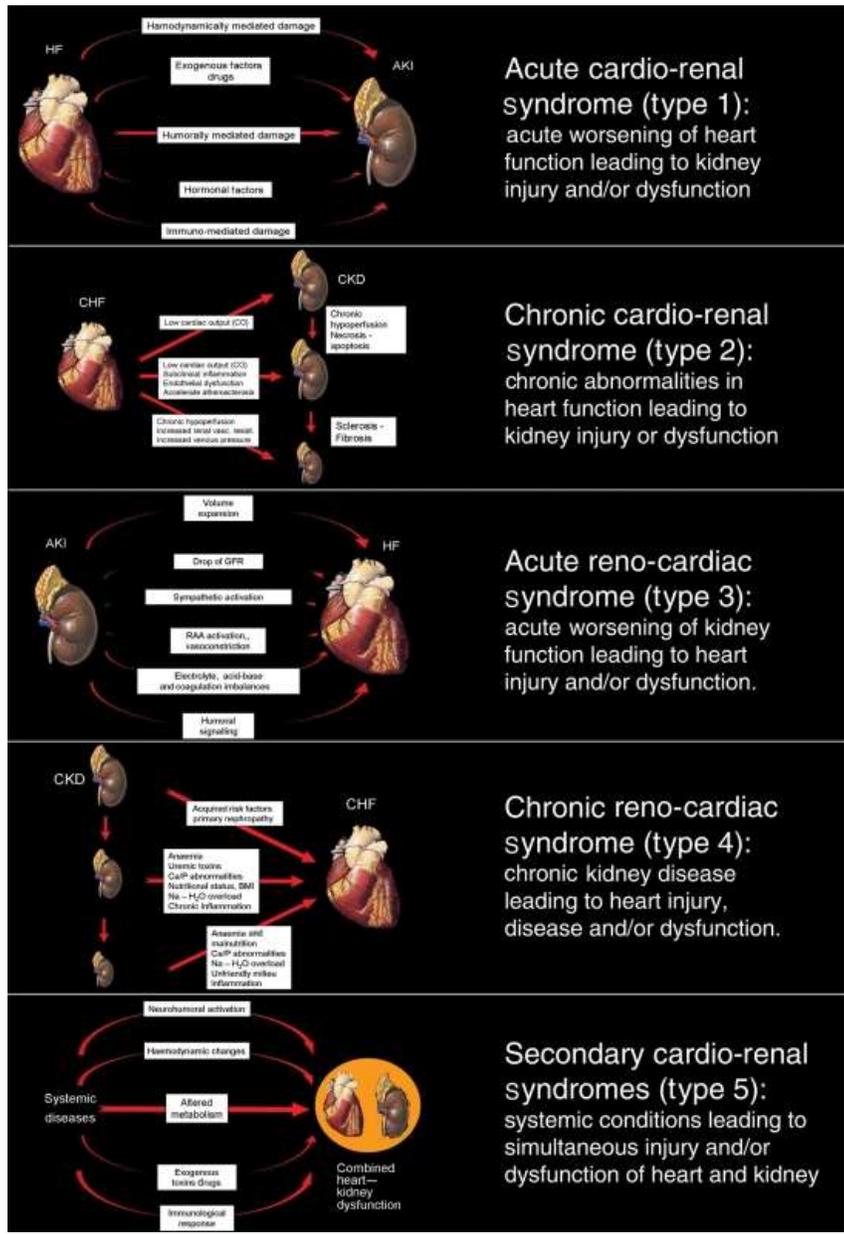
Different mechanisms are involved in the combined dysfunction of heart and kidney in these five types of the syndrome [Figure 2].

Type 5 CRS is defined as a systemic event which results in concomitant cardiac and renal dysfunction, such as sepsis. Actually, sepsis represent a prototypal condition that may cause an acute form of Type 5 CRS. Approximately 11-64% of septic patients develop AKI, and 46-58% have sepsis as a major contributing factor to development of AKI. Similarly, abnormalities of cardiac function are common in septic patients.

Coexisting acute kidney and myocardial dysfunction is, accordingly, common in sepsis, however there is a lack of integrative and epidemiologic studies that have specifically evaluated the

pathophysiology, incidence, risk identification, and associated outcomes for septic patients with concomitant AKI and myocardial depression who fulfil criteria for Type 5 CRS [16].

Figure 2: Pathophysiology and definitions of the five subtypes of cardio-renal syndrome



AIM

We conduct a series of studies to investigate the role of new technologies for clinical, instrumental and biochemical evaluation in ICU-AKI patients, with and without sepsis; we also analyzed which extracorporeal treatments can allow improve patients clinical pattern and outcome.

These studies aimed to:

- 1- Analyse the biochemical markers in AKI septic patients: Plasma NGAL for kidney injury, AOPP for OS and BNP for heart failure were evaluated and compare biochemical markers in AKI and No-AKI septic patients with ICU patients;
- 2- Analyse new methods to detect and manage fluid overload with non-invasive and minimally invasive techniques;
- 3- Compare Traditional versus Stewart approach to the detection of Acid-base disturbances in AKI patients undergoing CVVH;
- 4- Evaluated the role of the emerging extracorporeal treatments in the septic patients' population;

BIOCHEMICAL MARKERS OF AKI

Novel biomarkers beyond urine output and creatinine can help to make the diagnosis of AKI easier and earlier. An ideal biomarker should be sensitive and specific. Measurement should be technically easy with good reproducibility. Biomarkers levels should change in parallel with the degree of organ injury even in the absence of typical clinical signs and should enable early intervention.

Finally, the level of an ideal biomarker should correlate with both prognosis and response to treatment .

Neutrophil Gelatinase-Associated Lipocalin (NGAL): Human NGAL is a 25-kDa protein covalently bound to gelatinase and is one of the most up most upregulated transcripts in the kidney very early after acute injury: is a promising biomarker for early detection of AKI. Several studies have shown serum and urine NGAL to be useful early markers for AKI, particularly when the timing of the renal insult is known, such as post-cardiac surgery and radiocontrast exposure [17].

Advanced Oxidation Protein Products (AOPP): Critically ill patients are characterized by disordered vascular control, which is possibly initiated through the exhaustive production of reactive oxygen species (ROS) leading to reduction-oxidation reactions (redox) imbalance. An imbalance between production of ROS and production of antioxidants results in oxidative stress (OS). Measurements of ROS activity and damage due to OS could be a useful adjunct in the assessment of critically ill patients. Several studies have shown presence of advanced oxidation protein products (AOPPs) not only as a marker of OS but also as an inflammatory mediator, which may play an important role in the pathogenesis of acute diseases and acute and chronic renal failure [18].

Brain Natriuretic Peptide (BNP): is a cardiac neurohormone predominantly released from the ventricles in response to left ventricular volume expansion and pressure overload; levels of BNP are known to be elevated in patients with left ventricular dysfunction (LVD) and correlate with the New York Heart Association classification, as well as findings on echocardiography.

Although previous studies have shown that BNP levels are increased in patients with ESRD, these elevations are not caused solely by reduced renal clearance but appear to represent counterregulatory responses from cardiac ventricles. Thus, BNP may serve as an important plasma biomarker for cardiac stress and remodeling (ventricular hypertrophy) in patients with AKI and an useful biomarkers in CRS [19].

We investigate the possible correlation between serum biochemical markers of organ damage, such as Neutrophil Gelatinase-Associated Lipocalin (NGAL), Advanced Oxidation Protein Products (AOPP) and Brain Natriuretic Peptide (BNP) in ICU-AKI patients with or without sepsis.

Methods

SIRS was considered to be present when at least two of these criteria were met: temperature above 38°C or below 36°C, heart rate of more than 90 beats/min, respiratory rate of more than 20 breaths/min or partial pressure of carbon dioxide of less than 32mmHg, or white blood cell count above 12,000 mm³ or below 4,000 mm³. Sepsis was defined as SIRS plus infection.

AKI was defined using the creatinine and urine output criteria of the RIFLE classification.

Within four hours after admission blood was withdrawn for NGAL and BNP, with EDTA as an anticoagulant. Heparinized blood was withdrawn for AOPP evaluation.

Plasma samples for NGAL and BNP were quickly stored in minus 80 degrees Celsius for later analyzing. Plasma NGAL and BNP was measured with fluorescence-based immunoassay with the Triage point-of-care analyzer (Biosite Inc., San Diego, CA, USA) which is a rapid quantitative measurement of NGAL and BNP concentration in EDTA-anticoagulated whole blood or plasma.

AOPP were measured by spectrophotometry and calibrated with Chloramine-T solutions (Sigma Chemical Co., St. Louis, MO, USA) that in the presence of potassium iodide absorb at 340nm. Two hundred microliters of plasma diluted 1/5 in PBS and 20 µl of acetic acid were mixed and

calibrated versus the standard reference of 200µl Chloramine-T solution (0-100 µmol/L) with 20µl of acetic acid and 10 µl of potassium iodide.

The absorbance of the reaction mixture was read at 340nm against a blank containing 200µl of PBS, 10µl of potassium iodide, and 20µl of acetic acid. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents [18].

Differences between groups were analyzed using Student's t and Mann-Whitney tests as appropriate. Significant difference was accepted at $p < 0.05$.

Results

Ninety-eight consecutive adult patients admitted to ICU of San Bortolo Hospital, Vicenza, Italy, between October 2008 and August 2010, were enrolled in this study. Patients were divided in two groups depending on the presence of sepsis, defined as Systemic Inflammatory Response Syndrome (SIRS) associated with an infectious process. Fifty-six patients had sepsis, while forty-two patients were non septic. Among septic patients, twenty-four subjects developed AKI, defined by RIFLE criteria, while thirty-two did not. AKI occurred in fourteen patients without sepsis as well.

Ninety-eight consecutive adult patients admitted to ICU, divided in two groups depending on the presence of sepsis were enrolled.

In these patients the levels of NGAL, BNP and AOPP were significantly higher among septic patients compared with non septic subjects ($p < 0.001$).

Among septic patients, subjects who developed AKI showed significant higher levels of NGAL, AOPP ($p = 0.0425$), and BNP ($p = 0.0327$).

FLUID STATUS MANAGEMENT OF AKI

Several studies shown that a positive fluid balance was an important factor associated with increased 60-day mortality. Volume assessment and management in critically ill patients remains challenging. Issues of timing, choice, amount of fluids, and type of volume assessments to guide therapy continue to be investigated. While early volume resuscitation and goal-directed therapy have been shown to improve mortality and morbidity and lessen the risk of acute kidney injury, management of patients with established acute lung injury, AKI or both (as well as in sepsis) kidney injury reveals that a more conservative or “dry” strategy is more appropriate than a liberal or “wet” one [20]. Studies of the assessment of fluid status have shown that simple central venous pressure (CVP) monitoring is as effective, and safer, than more invasive means such as pulmonary artery occlusion pressure. It is clear, however, that CVP does not tell the entire story, as patients with high right sided pressures may have reduced, normal, or increased effective circulating volume. Bioimpedance vectorial analysis (BIVA) allows determination of extracellular fluid volume and total body water from measurements of resistivity of tissues to single or multifrequency emitted signals. BIVA has been used to manage volume in hemodialysis patients for several decades [21].

However, the use of BIVA in critically ill patients has not been extensively studied, and the data used to determine volume status have been derived from hemodynamically stable patients. BIVA could be useful the assessment of tissue hydration status in critically patients.

Brain natriuretic peptide (BNP) is a biomarker used to identify patients with fluid overload and congestive heart failure. In critical care, it has been shown to correlate with mortality and morbidity, though it has not been used to guide therapy [22].

We conducted a pilot study to examine the relationships between CVP, BIVA, and BNP in order to determine which measure, or combination of measures, relate to volume status in critically ill, ventilated patients.

Material and Methods

This study was approved by the Institutional Review Board of the San Bortolo Hospital, Vicenza, Italy, and conducted in the Intensive Care Unit (ICU). Adult AKI patient requiring mechanical

ventilation was eligible. Because of the technical requirements for BIVA, patients with any upper or lower limb amputation, severe rhabdomyolysis, or erysipelas of both upper or lower limb were excluded. As the study required serial measurements over time, any patient not expected to survive 72–96 hours was excluded. Any patient with recent cardiac surgery was also excluded, as BNP and CVP may be grossly

skewed. Likewise, patients with decompensated heart failure or acute coronary syndrome were excluded. As published, BIVA vectors were derived in Caucasians, we excluded non-Caucasians. Within 48–72 hours of initiating mechanical ventilation, baseline assessment was undertaken including CVP, BIVA, and blood sample for BNP, hematocrit, and creatinine. BNP was determined using Triage MeterPro (Biosite Inc., San Diego, CA). CVP and BIVA were recorded in a blinded fashion by separate trained observers. CVP was obtained through a central venous catheter connected to a calibrated transducer using the level of the right atrium as a reference point. BIVA was performed using a plethysmograph emitting 800- μ A and 50-kHz alternating sinusoidal current (EFG

Electrofluidgraph, Akern s.r.l., Pontassieve, Florence, Italy) and previously published methods.

Clinical data were recorded, including primary illness, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), weight, urine output, pressor doses, PO₂, FiO₂, and mean airway pressure. The fluid balance in the intervening period was calculated.

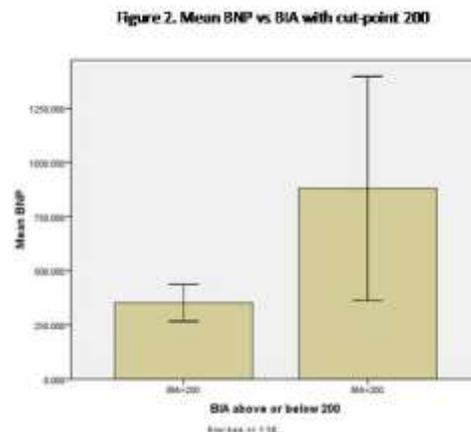
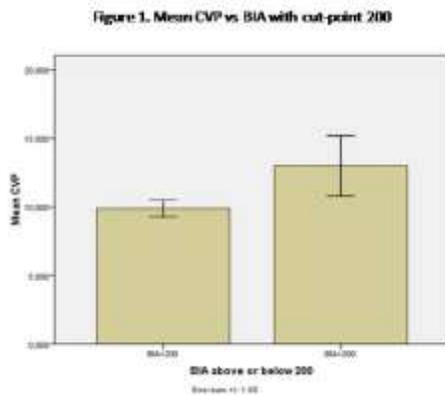
CVP was categorized as “low” (<4 cm H₂O), “high” (\geq 14 cm H₂O), or “normal” (4–14 cm H₂O).

For the BIVA, three patterns were considered according to published references for resistance/height (R/H) based on normals, adjusted for age, sex, and weight: long vectors outside the 75% tolerance ellipse (upper pole of the target) were categorized as “dehydrated” and short vectors outside the 75% tolerance ellipse (lower pole of the target) as “hyperhydrated”, while the remainder were “normohydrated”; Using the percentage of extracellular water (ECW) obtained by BIVA, patients were classified into 3 groups; over-, normo- and underhydrated. In addition, patients were also classified by resistance(Rz) / height(H) ratio (Ω /m): $>200\Omega$ /m = dry, $\leq 200\Omega$ /m = wet .

The agreement between slopes of CVP, BIVA, and BNP against O2I were estimated using Kappa statistics. Univariate correlations were assessed at baseline for CVP, BIVA, and BNP. Slopes of change were also assessed between these variables and hemodynamic parameters, fluid balance, and other clinical parameters such as hematocrit and creatinine.

Results

The baseline characteristics are as follows: 34 patients, mean SOFA score 7.1 ± 0.4 ; CVP 10.4 ± 0.6 mmHg; BIA (Rz/H) 262 ± 14 Ω /m; BNP 451 ± 119 pg/mL; creatinine 1.36 ± 0.22 mg/dL; MAP 89.9 ± 2.8 mmHg. Patients categorized by Rz/H as wet showed a weak but clinically congruent association with a higher CVP (13.0 vs 9.9 mmHg, $p=0.065$)[Fig.1] and higher BNP (882 vs 352 pg/ml, $p=0.083$) [Fig.1-2].



We conclude that BIVA can be considered an extra tool to assess overall fluid status of the patient and to help optimize fluid management.

STROKE VOLUME VARIATIONS

Fluid administration in critically ill patients is typically performed to increase cardiac preload, followed by a raise in cardiac output. However, studies conducted during the past few years have shown that about 50% of critically ill patients do not exhibit the desired effect (they are not fluid responsive), particularly in AKI populations, that with a non-effective fluid responsiveness has a higher risk of fluid overload and need of renal replacement therapies.

Stroke Volume Variations (SVV) is a dynamic cardiac preload parameter, and its wide variations are described in the literature as a predictor of volume responsiveness in several populations of mechanically ventilated patients [23].

AAA surgery patients are at high risk for AKI, which may be due to hypovolemia, haemorrhage, decreased cardiac output, vascular disruption, or inflammation.

We conducted a pilot study in patients who undergo surgical interventions of abdominal aortic aneurysm (AAA) surgery with supra-renal clamping to assess if wide variations of SVV before and after clamping of the aorta are correlated with a higher risk of AKI development than those with lesser variations.

Methods

Patients were all mechanically ventilated and fully controlled with volume-control mechanical ventilation, tidal volumes of 8 ml per kg of predicted body weight, inspiratory:expiratory ratio of 1:2, and positive end expiratory pressure of 4 cmH₂O. Patients with sustained arrhythmias, spontaneous ventilations, and those extubated before 12 h post-operative were excluded. SVV was measured with the FlowTrack/Vigileo(Edwards®) device every 3 minutes during the procedure and for 48 h after surgery. A detailed log of ins and outs was kept. Patients received 70ml/kg/h of crystalloids, and fluid boluses and transfusions were given if needed according to medical team preference. Data was compared with non-parametric tests.

SVV was calculated from percentage changes in SV during the ventilatory cycle. SV is assessed by the FloTrac™/Vigileo™ and Briefly, calculation of SV by the FloTrac™/Vigileo™ system is based on the contribution of pulse pressure to SV being proportional to the standard deviation of

arterial pulse pressure. In order to determine SV, the influences of vascular resistance and compliance on SV are considered using manually entered patient data and pulse wave analysis.

In all patients SVV was measured with the FlowTrack/Vigileo device by experienced anaesthesiologists and intensivists immediately after endotracheal intubation and after aortic clamp time, every 15 minutes during the procedure, immediately after declamp time and surgical closure, and 24 hours after surgery.

Results

Out of 8 patients, 4 developed AKI in the first 12 post-operative hours. As compared to patients without AKI, AKI patients had a significantly larger SVV after aortic clamping (13.25 vs. 24.5, $p=0.01$). The increase in SVV at aortic declamping time, as compared to SSV at clamping time, was also significantly higher in AKI patients (-3.75 vs. 12.5, $p=0.04$) [Fig.1-2].

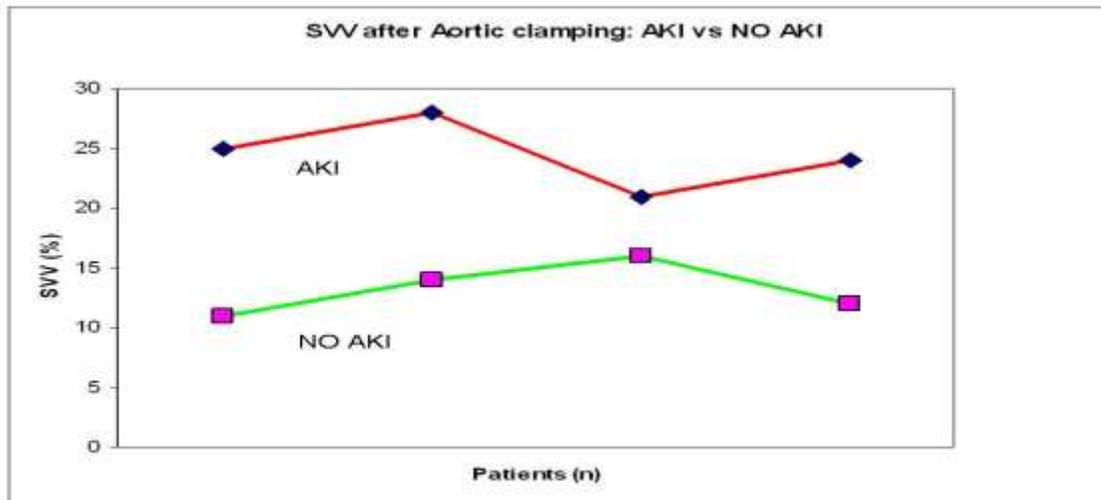


Fig. 1 AKI patients had a significantly larger SVV after aortic clamping (13.25 vs. 24.5, $p=0.01$).

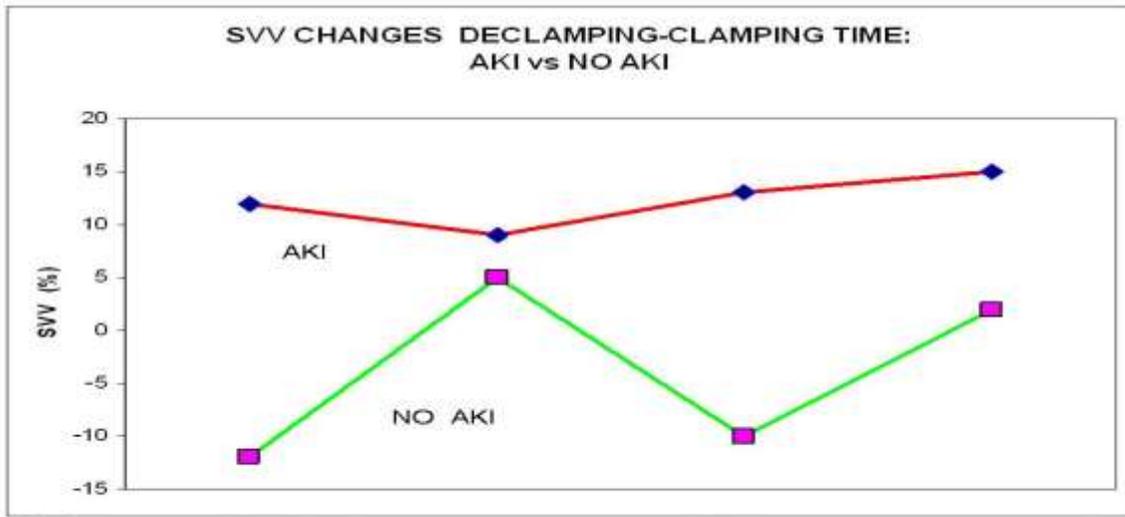


Fig. 2 The increase in SVV at aortic declamping time, as compared to SSV at clamping time, was also significantly higher in AKI patients (-3.75 vs. 12.5, $p=0.04$).

SVV monitoring during and after suprarenal AAA surgery is a feasible tool that can help identify patients at risk for AKI development.

MANAGEMENT AND DETECTION OF ACIDOSIS

Acidosis it's one of the most commonly seen problems in critically ill patients and represent a common indications for RRT in ICU. The best approach for acid-base analysis is debated.

Classical approaches use Henderson Hasselbach variables with measured and derived parameters and Anion gap (AG) calculation. Siggard-Andersen approach includes the calculation of Base Excess (BE)

and Standard BE (SBE).

Physical-chemical approach apply Stewart's method: each one of these methods has several limitations in the ICU setting [24].

For critically ill patients the Stewart approach may be better than the Classic approach

AIM

Assessment of correlation and agreement between classical versus Stewart approaches for the analysis of acid-base disturbances before and after 6,12, and 24 hours of CRRT in Intensive Care Unit (ICU) patients on MV.

Materials and Methods

We conduct a prospective cohort study All the patients admitted to the ICU with AKI that required RRT were included in the study.

All patients were treated with CVVH with a LYNDA[®] machine, (Bellco, Mirandola, Italy), with a blood flow rate of 200-250 ml/min and with a prescribed infusion rate of at the least 35 ml/Kg/hr. All treatments were performed with polyetersulfone 1.7 m² dialyzer (DIAPES, Bellco, Mirandola, Italy). We use a standard buffer (5 Liters: Bicarbonate 35 mmol/L, sodium 140 mmol/L, Potassium 2 mmol/L, Calcium 1,75 mmol/L, magnesium 0.5 mmol/L, PH 7,4). Demographic and clinical data were collected. The Acute Physiology and Chronic Health Evaluation (APACHE) II and Sepsis related Organ Failure Assessment (SOFA) [26] scores were calculated on admission. Organ dysfunction was defined as a SOFA score [2 points in the organ systems evaluated—cardiovascular, respiratory, neurological, hematological, renal and hepatic].

The samples were collected before CVVH start, and after 6, 12 and 24 hours of CVVH. Blood gases were analyzed to measure pH, PaCO₂ and bicarbonate (HCO₃⁻).

A venous blood sample was analyzed to measure electrolytes, lactates, plasma protein, and albumin

Bicarbonate and SBE were calculated using the Henderson- Hasselbach and Siggaard-Andersen equations.

All patients received a 35 ml/Kg/h infusion of a standard buffer [5 Litres (mmol/L): [HCO₃⁻]35, [Na⁺]140, [K⁺]2, [Ca²⁺]1,75, [Mg⁺]0.5, pH 7,4]. We calculated [HCO₃⁻] and SBE with the Henderson- Hasselbach and Siggaard-Andersen equations.

Based on the Standar Base Excess (SBE) , the metabolic status of the patients was classified as acidosis (SBE -5.0), normal (SBE between -4.9 and 4.9) or alkalosis (SBE +5.0). The Anion Gap (AG) was calculated by the standard formula: $AG = [Na^+] + [K^+] - [Cl^-] - [HCO_3^-]$, with an elevated AG defined as greater than or equal to 17 mEq/L. The corrected AG was also calculated (AGcorr) to compensate for the patient's albumin, phosphate and lactate concentrations, using the formula: $AG_{corr} = AG - (2[albumin \text{ in g/dL}] - 0.5[phosphate \text{ in mg/dL}]) - lactate$. Physicochemical analysis was performed using the Stewart equations modified by Figge et al. to consider the effects of plasma proteins.

First the Apparent Strong Ion Difference was calculated (SIDa): $SIDa = [Na^+] + [K^+] + [Mg^{2+}] + [Ca^{2+}] - [Cl^-] - [lactate]$ (all concentrations in mEq/L). The normal variation for SIDa was defined as 40–42 mEq/L. The Effective Strong Ion Difference (SIDE) was then calculated. This equation considers the contribution of plasma weak acids (PaCO₂, albumin and phosphate) to the electroneutrality of plasma. The formula for SIDE, as determined by Figge et al., is $SIDE = 1000 \times 2.46 \times 10^{-11} \times PaCO_2 / (10^{-pH}) + [alb] \times (0.123 \times pH - 0.631) + [phosphate] \times (0.309 \times pH - 0.469)$. The normal variation for SIDE was defined as 38–42 mEq/L. The difference between SIDa and SIDE is expressed as strong ion gap (SIG) by utilizing the formula $SIG = SIDa - SIDE$. An elevated SIG value SIG was defined as > 2 mEq.

Chi square test was used at 0, 6, 12 and 24h after CVVH to compare acidosis detected with pH and SIDE and BE vs SIDa

Spearman-rank Correlation Coefficient was used to evaluate the relationship between pH, SBE, HCO₃⁻ and SIDE values after 24h of CVVH and 1-month mortality.

Results

We enrolled 19 consecutive adult patients on CVVH and mechanical ventilation. the average age was 58 years, AKI stage III (48.6 %) and stage II (38.3%) were most prevalent.

ICU length of stay was 22 days and icu mortality rate was 57.9%, respiratory failure and septic shock were the two most common diagnosis at time of their ICU admission.

The prevalence of acidosis after CVVH, as assessed by pH vs. SIDe, was [36.8% vs. 94.7% (p<0.001)] at 6h, 21.1% vs. 73.7% (p<0.05)] at 12h, and [21.1% vs. 98.6% (p<0.001)] at 24h.

The prevalence of acidosis after CVVH, as assessed by SBE vs.SIDe, was [57.9% vs.94.7%(p<0.05)] at 6h,[63.2% vs.73.7% (p=NS)]at 12 h,and [63.2% vs.98.6%(p<0.05)] at 24h [Fig. 1-2].

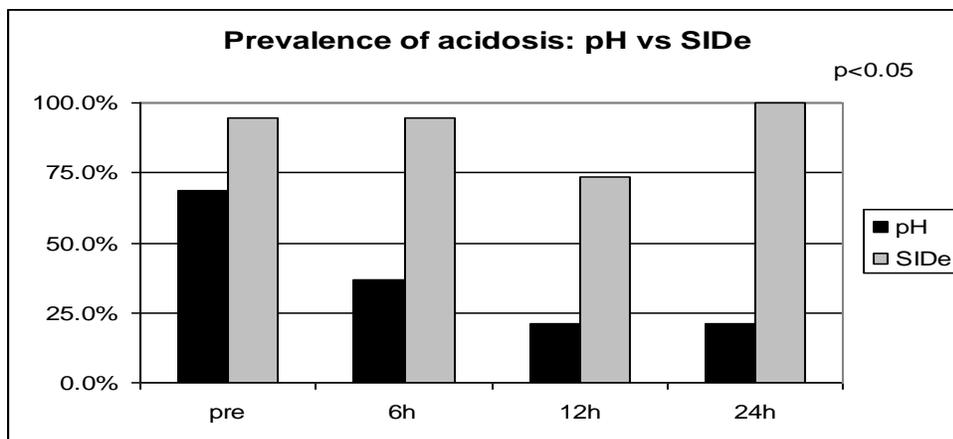


Fig1. Prevalence of Acidosis: pH vs SIDe.

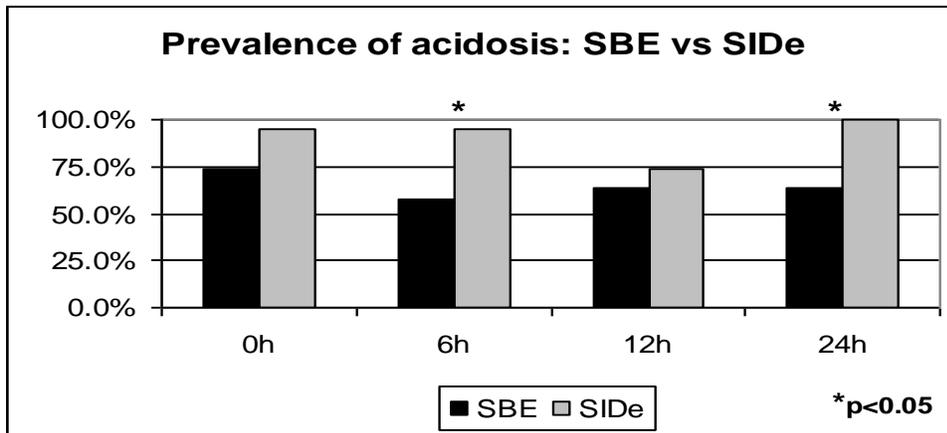


Fig2 . Prevalence of Acidosis: SBE vs SDe.

Patients who survived had significantly higher levels of SDe compared to patients did not survive.

No significant difference were found between patients who survived and patients who did not survive by pH, HCO₃ and SBE.

Stewart approach seems to be more sensitive than Classical approach for detection of acidosis in ICU patients on CRRT.

NEW EXTRACORPOREAL TREATMENTS FOR AKI AND SEPSIS

Acute Kidney Injury (AKI) in the Intensive Care Unit (ICU) has been shown to be a significant risk factor for mortality, particularly when patients need renal replacement therapy (RRT).

Actually we have a couple of extracorporeal treatments (Tab.2): such technologies can remove mediators via convection [25], diffusion, or adsorption[26], and may provide organ support, the modulation of inflammatory mediators with additional physiological benefits such as temperature control, acid-base control, fluid balance control, cardiac support, protective lung support, brain protection with preservation of cerebral perfusion, improved bone marrow function, blood detoxification, and liver support [27-29].

High Volume Hemofiltration (HVHF) and Coupled Plasma Filtration Adsorption (CPFA) have shown potential improvements in septic animals, but actually there are no studies that compare these two types of treatments in human.

HVHF its a particular hemofiltration with a large use of replacement fluid (more than 50 ml/kg/h); P(pulse) HVHF (pHVHF) is the combination of a HVHF performed for 6 to 10 hours followed by standard CVVH for the rest of the day. Both modalities are usually performed in pre-dilution, although post-dilution can be added (20-40%) [30].

Coupled plasma filtration adsorption (CPFA) is an extracorporeal therapy that uses a plasma filter to separate plasma from the blood; the separated plasma then passes through a sorbent cartridge for the nonspecific removal of various mediators. The plasma is returned to the blood after purification. This blood can then pass through a hemodialyzer/hemofilter for additional blood purification by conventional hemodialysis, hemofiltration or hemodiafiltration in patients with acute renal failure (ARF) [Fig. 3] [31].

Table 2: Current Extracorporeal Treatments modality in Critically ill

	TYPE OF TREATMENT	ORGAN SUPPORT	MECHANISM
HEMODIALYSIS	INTERMITTENT	KIDNEY	DIFFUSION
SLED-EDD <i>(Sustained Low Efficiency Dialysis or Extended Daily dialysis)</i>	INTERMITTENT	KIDNEY	DIFFUSION
CVVH <i>(Continuous Venous-Venous Hemoiltration)</i>	CONTINUOUS	KIDNEY	CONVECTION
CVVHD <i>(Continuous Venous-Venous HemoDialysis)</i>	CONTINUOUS	KIDNEY	DIFFUSION
CVVHDF <i>(Continuous Venous-Venous HemoDiaFiltration)</i>	CONTINUOUS	KIDNEY	CONVECTION AND DIFFUSION
HVHF <i>(High Volume HemoFiltration)</i>	CONTINUOUS OR INTERMITTENT (pulse HVHF)	KIDNEY, HEART LUNG IMMUNE SYSTEM	CONVECTION
IHD or CVVH WITH HIGH PERMABILTY MEMBRANE	CONTINUOUS OR INTERMITTENT	KIDENY, IMMUNE SYSTEM	SOLUTE REMOVAL WITH HIGH CUT OFF MEMBRANE
CPFA® <i>(Coupled Plasma Filtration Adsorption)</i>	I INTERMITTENT	KIDNEY HEART LUNG IMMUNE SYSTEM	PLASMA ADSORPTION AND CONVECTION or DIFFUSION
TORAYMIXIN®	INTERMITTENT	IMMUNE SYSTEM	ADSORPTION (ENDOTOXIN REMOVAL)
PROMETHEUS® MARS® (Molecular Adsorbents Recirculation System)	I NTERMITTENT	LIVER	PLASMA DETOXIFICATION AND ALBUMIN DIALYSIS
DECAP®	INTERMITTENT	LUNG KIDNEY	CO2 REMOVAL AND CONVECTION
RAD <i>(Renal Assist Device)</i>	INTERMITTENT	KIDNEY	CONVECTION AND TUBULE FUNCTION

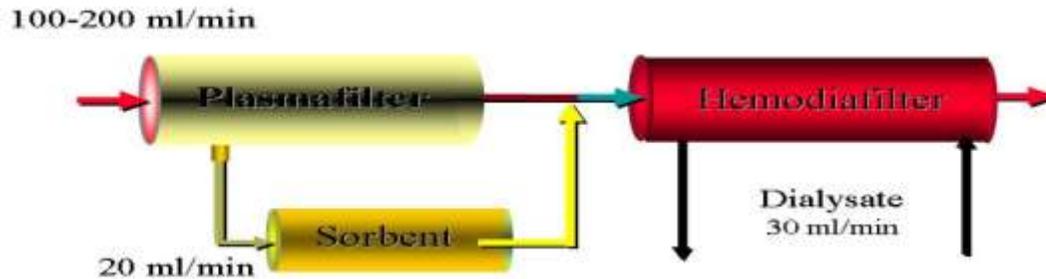


Fig. 3: CPFA circuit

The treatment goal of CPFA is to target the excess of circulating mediators in order to counteract the disproportionate pro and anti-inflammatory reaction which occurs in sepsis.

Our aim was to compare the hemodynamic effects of HVHF and CPFA in septic shock patients with Acute Kidney Injury (AKI) undergoing Continuous Renal Replacement therapies (CRRT).

Materials and Methods

We performed a cross-over study enrolling patients with septic shock and AKI who were receiving CRRT. Patients were treated with pulse HVHF (pHVHF) and CVVH on Day 1 and CPFA and CVVH on Day 2 or vice versa. pHVHF was performed for 8-10 hrs with replacement fluid rate of 85 ml/kg/hr. CPFA was performed for 8-10 hrs with a plasma flow rate of 15%. CVVH was performed for the rest of the day with replacement fluid rate of 35 ml/kg/hr. The primary endpoints were changes in mean arterial pressure, vasopressors requirement (expressed as *Vasopressor Score*, VS) and changes in noradrenaline dose, changing in the SOFA score and changing in Organ Dysfunction

after pHVHF and CPFA. Secondary endpoints are related to leukocyte cell function (Monocyte %, MFI and HLA-DR+ expression) and Oxidative stress (AOPP). The two treatments were compared using nonparametric tests.

Results

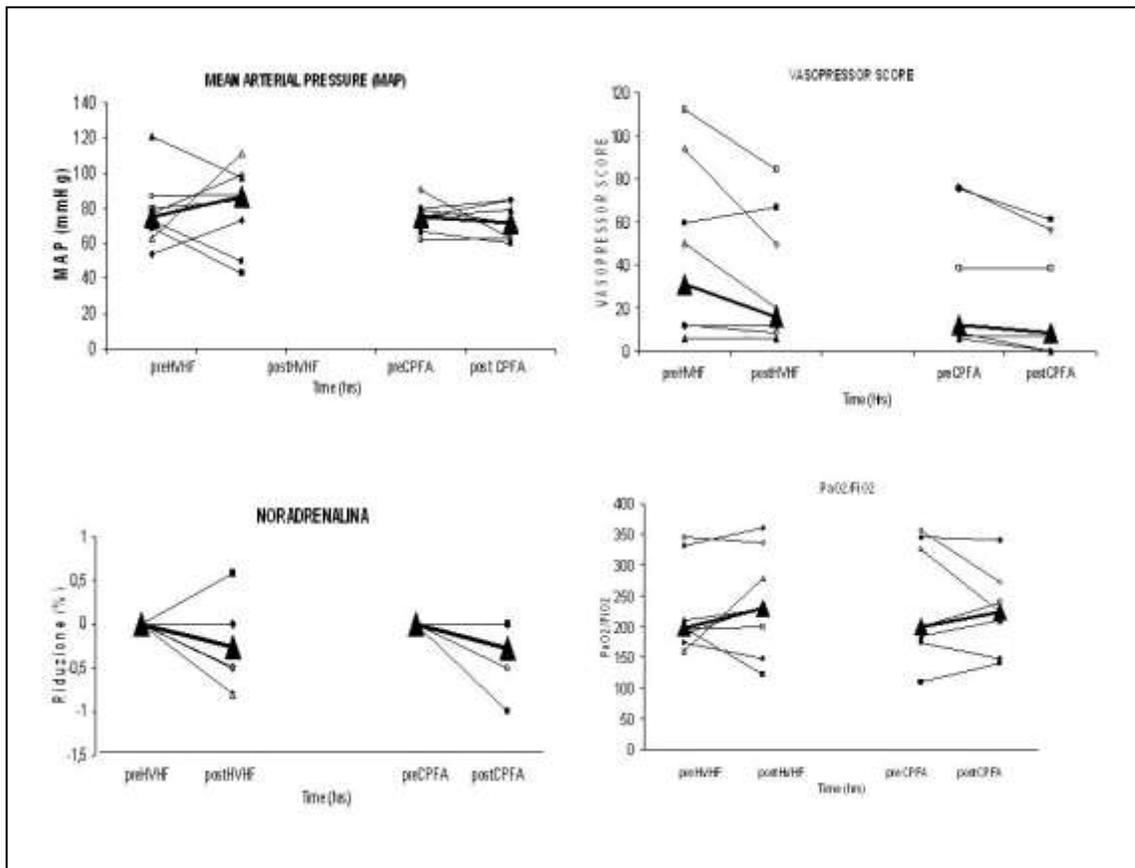
We enrolled 8 patients (median age 70.5 yr, SOFA 12.5, SAPS II 69.5). There was a trend for reduction in VS with HVHF and CPFA (HVHF $p=0.13$, CPFA $p<0.05$) There was not a significant difference between the two treatments in terms of percentage change in VS score ($p=0.22$) [Fig. 4].

Others results are showed in Table 3

HVHF appears more useful in depuration, reduction of vasopressors requirement and seems to be more useful to improve PaO₂/FiO₂ ratio.

CPFA reduce Vasopressor Score and AOPP concentration.

Fig 4: MAP, Vasopressor Score (VS), Dose di Noradrenalina e rapporto PaO2/FiO2.



	Pre-pHVHF	Post-pHVHF	p	Pre-CPFA	Post-CPFA	p	pHVHF vs CPFA p
MAP (mmHg)	75 (67.5-81.75)	86.5 (67.2-98.2)	0.88	75 (71-79.5)	72 (63-82.1)	0.73	0.29
PaO2/FiO2	197 (183.6-240.6)	230 (186.7-292.6)	0.48	198.6 (178.9-335.3)	231.5 (166.1-263.8)	0.07	0.08
Noradrenalina (mcg/kg/min)	0.2 (0-0.5)	0.07 (0-0.4)	0.13	0.07 (0-0.04)	0.03 (0-0.3)	0.06	0.18
Vasopressor Score (VS)	30.9 (11.4-68)	15 (10-53.8)	0.13	12 (7.5-56.6)	8.3 (3.3-47.3)	0.04	0.22
SOFA Score	12.5 (10-13)	11 (9-11.5)	0.60	10 (8.5-12.5)	10 (10-11)	0.66	0.68
Creatinina (mg/dL)	1.9 (1.2-1.5)	1.2 (0.8-1.5)	0.01	1.4 (0.9-1.7)	1.4 (0.9-1.6)	0.86	0.01
Urea(mg/dL)	88.5 (83.7-106.5)	54 (44.7-57.2)	0.01	54 (50.2-74.3)	36.7 (36.1-37.2)	0.86	0.01
Lattati (mmol/L)	2.5 (2-2.4)	2 (1.8-2.2)	0.33	2 (1.6-2.2)	2 (1.9-2)	0.27	0.44
HLA-DR+ (%)	41.5 (34.6-54.5)	50.2 (38.3-56)	0.45	43.2 (36.3-65.8)	34.8 (31.9-64.7)	0.63	0.09
AOPP (nmol/L)	199.9 (106.2-293.5)	234 (193.1-342.5)	0.08	164 (139-243.8)	142 (118.4-193.3)	0.09	0.06

Table 3: Results for primary and secondary end-points.

The data from this pilot study provide no evidence for a difference in hemodynamic effects between pHVHF and CPFA in patients with septic shock already receiving CRRT. A larger sample size is needed to adequately explore this issue.

DISCUSSION

Sepsis is one of the most common causes of death in the ICU and mortality is even higher when associated with AKI.

The last decade has witnessed many advances in the treatment of AKI. However, morbidity and mortality have not improved significantly.

Serum creatinine is an insensitive and late marker for AKI. It is important to diagnose AKI as early as possible to facilitate effective interventions.

Biomarkers are biological substances of human physiology, reflecting change in function or appearance of injury of a certain organ or system of organs. The ideal biomarker is easily measured, specific for the organ under inspection, appears early after injury, shows the amount of injury, and correlates with prognosis [32].

NGAL has emerged as a novel biomarker of AKI. NGAL is a 25-kDa protein widely spread within human body (kidney, prostate, uterus, salivary gland, epithelia of respiratory and alimentary tracts), and shown to possess various biological properties, for instance kidney-protecting and nephron-inducing activity and bacterio-static capability. Although normally expressed at very low levels, it has been shown to rise in AKI, in human kidney cortical tubules, urine and plasma, and has therefore become a novel biomarker of acute renal damage. Furthermore, the rise of NGAL takes place faster than a possible increase in plasma creatinine allowing detection of AKI earlier than with creatinine-based criteria and with good sensitivity and excellent specificity. NGAL has been shown to increase in various settings of cardiac procedures and in critically ill patients with sepsis, renal ischemia and contrast-media induced nephropathy. The acute rise of NGAL in most reported studies has taken place during 2-6 hours after an event compromising renal function and causing renal damage.

According to other published data NGAL levels were significantly higher in AKI septic patients compared to No-AKI septic patients, and higher in AKI septic patients compared to AKI ICU patients. The concomitant presence of sepsis and AKI causes an increase of NGAL; this is probably due to infections that renal damage [32-33].

Moreover, in AKI septic patients both NGAL and BNP were higher than other groups of patients. Sepsis probably caused a cardiac-renal damage by increasing levels of biomarkers.

An increase in OS is typically present in critically ill patients as a consequence of the overproduction of Reactive Oxygen Species (ROS) and of a rapid depletion of the endogenous stores of antioxidants.

OS has been incriminated in the pathogenesis of the systemic inflammatory response and organ dysfunction, via cellular energetic failure and via an interaction with several pathways after lipid peroxidation, and oxidative damage to proteins, DNA, and RNA.

Several potential causes might contribute to extra-cellular and intra-cellular augmentation of oxidant stress in these patients: the high inspiratory concentrations of oxygen required to achieve adequate arterial oxygenation, activation of neutrophils, pro-oxidant drugs, or systemic infections all can promote ROS accumulation and consumption of anti-oxidative factors.

In physiologic conditions, increased OS is desirable for some cell functions (proliferation, gene expression, apoptosis). The role and importance of the ROS in the regulation of these functions during critical illness is only partially understood.

Several studies have evaluated OS in critical illness: Himmelfarb et al [34] previously demonstrated that OS, as reflected by decreased thiol content and elevated carbonyl content, is higher in critically ill patients with acute renal failure (ARF), as compared with healthy controls, end-stage renal disease patients, and ICU patients without ARF.

In 2010 Lentini, de Cal et al. [35] measured AOPP for 4 days in patients and therefore they were able to appreciate the fluctuation of OS over time. This study was also the first one to assess the correlation between OS and the degree of severity of AKI: by showing that patients with AKI have significantly elevated AOPP levels, as compared with No-AKI patients this study confirmed previous findings related to elevated OS in AKI, but interestingly, they demonstrated that patients with the most severe AKI (RIFLE class Failure) had markedly elevated AOPP levels compared with all other patients, whether No-AKI or RIFLE R-I AKI patients.

It also remains unclear if elevated OS is simply an epiphenomenon, or also causative for AKI.

Moreover, in AKI septic patients levels of AOPP were very high, while, in ICU patients without AKI, levels were lower.

BNP have gained success as diagnostic and prognostic biomarkers, especially among CHF patients. BNP is secreted by the cardiac ventricles in response to excessive stretching of myocytes, in heart failure and volume overload, and ischemic injury to myocardium. In our

results the levels of BNP were significantly higher in septic patients compared with ICU patients, and moreover in AKI septic patients compared with No-AKI septic patients[36].

Importantly, elevated levels of BNP are independent predictors of cardiovascular morbidity and mortality, both in patients with normal and impaired renal function, thus emphasizing the value of BNP in assessment of CRS[36]. We also found elevated level of BNP in AKI patients with Fluid Overload.

Volume Status is one of the most difficult topic in critical ill and fluid overload, as reported in the SOAP study seems to be directly related to mortality[37].

Fluid administration is frequently necessary to stabilize the patient with severe sepsis or septic shock. Moreover, sepsis has been reported to account for approximately 50% of patients with acute kidney injury (AKI) in ICU. Thus the timing and type of fluid administered as well as renal outcomes are very important. Recently, the timing of the fluid administration and the physiologic end points to be monitored have emerged as Early Goal-Directed Therapy (EGDT) [38]. In the patient with septic shock, early intervention with fluid resuscitation within the first 6 hours in the emergency department (ED) has been described as EGDT.

Previous studies had generally reported results relating to later interventions in ICUs after admission from the ED.

On the other hand, recent observational studies have shown a correlation between fluid overload and mortality in AKI patients whether or not they necessitated dialysis. Moreover, the Adult Respiratory Distress Syndrome (ARDS) network performed a randomized study in critically ill patients to compare liberal *versus* conservative fluid administration [39]. The liberal fluid administration group exhibited worse pulmonary function and no protection of renal function. Constancy of central venous pressure (CVP) measurements in the 12-mmHg range were observed in the liberal fluid group despite a mean increase in positive fluid balance of 7 L, thus suggesting increased interstitial fluid accumulation leading to pulmonary congestion[40].

We conducted a pilot study to examine the relationships between CVP, BIVA, and BNP in order to determine which measure, or combination of measures, relate to volume status in critically ill, ventilated patients.

Bioimpedance vectorial analysis (BIVA) allows determination of extracellular fluid volume and total body water from measurements of resistivity of tissues to single or multifrequency emitted signals.

With CVP (intracellular fluids), BIVA (extra cellular fluids) and BNP we can attempting to find a combination of minimally invasive bedside tools for volume assessment in critically ill patients.

We identified that changes in CVP and BNP over time were correlated with important changes in BIVA[41].

It is intuitive to think that measures of volume, be they measures of intravascular or extravascular (interstitial) volume, would be related to lung function and oxygenation.

For instance, animal studies have shown that fluid balance can influence both the onset and resolution of severe “highpermeability” pulmonary edema. Excess extravascular lung water is a feature of all types of pulmonary edema, and lower extravascular lung water correlates with fluid balance, decreased ventilator days, and ICU length of stay. We chose CVP since local practice was such that most mechanically ventilated patients had central venous access appropriate for measurement of CVP. Furthermore, studies indicate a high level of agreement between clinical measures such as the external jugular pressure and the CVP; hence, a ready estimate of CVP would be available in all subjects. We chose BIVA for its ease of use and noninvasive nature and its ability to provide an estimate of extravascular water. BNP was chosen for its ability to respond to myocardial stretch and its utility in previous studies as a predictor of outcome.

Previously Piccoli and colleagues [42] demonstrated a degree of inverse correlation between CVP and impedance vector components, though this was stronger in the group that had significantly elevated CVP and weaker in the group with lower CVP. These authors suggested that the combination

of CVP and BIVA might be useful in the volume assessment and management of critically ill patients. We strongly confirm this advice . The principle difference between our study and that of Piccoli is that

the minority of patients in the latter study were receiving mechanical ventilation, while this was a requirement for eligibility in our study. This may have played a role since mechanical ventilation with positive end-expiratory pressure (PEEP) likely and systematically elevated the CVP and

possibly weakened any potential relationship between CVP and BIVA. Moreover, all the patients in our study had relatively high values of PEEP (8–10 cmH₂O).

We were able to demonstrate that change in BNP was associated with change in BIVA and this relationship was modestly in multivariable regression by including slope of change of CVP.

Another limitation of the study is that the methods used to assess volume were not compared against other methods such as echocardiography, ultrasound of the inferior vena cava, pulse pressure variation, or stroke volume variation. This is a fair criticism; however, the study presented is the first in a series of pilot endeavours, the intent of which is to examine varying combinations of volume assessment.

Thus, we require an accurate and reliable technique to guide fluid management. Pressure preload variables (central venous pressure and pulmonary capillary wedge pressure), which continue to be used, often fail to provide reliable information regarding cardiac preload and are incapable of predicting cardiac response to fluid therapy. On the other hand, the volumetric preload

variables that are assessed by transpulmonary thermodilution may better reflect left ventricular preload, but they do not allow assessment of fluid responsiveness[43].

As an alternative to these static variables, a dynamic approach may be used in the form of preload monitoring to guide fluid therapy. Different, less invasive haemodynamic monitoring systems based on arterial pulse contour analysis allow stroke volume variation (SVV) to be tracked continuously

In a cohort of elective patients who were underwent AAA surgery we perform an additional studies utilizing the FloTrac Sensor and Vigileo Monitor (Edwards Lifesciences, S.A., Saint-Prex Switzerland) to measure stroke volume variation.

The FloTrac/Vigileo™ system assesses SV typically using signal detection via a peripheral radial artery. It analyzes the impact of vascular tone on pressure during a period of 20 seconds, and adjusts

for actual vascular tone at intervals of 1 minute based on wave form analysis and patient characteristics.

In our pilot study patients with AKI had a large variations of SVV after aortic clamping and the increase in SVV at aortic declamping time, as compared to SSV at clamping time, was also significantly higher in AKI patients. These effects seem to be related to an abrupt, acute reduction of renal perfusion, able to give “renal angina” and AKI; SVV monitoring during and after suprarenal AAA surgery is a feasible tool that can help identify patients at risk for AKI development. Particularly, an increase in SVV at aortic declamping time, as compared to SSV at clamping time, may be a marker for risk of AKI development that should be further studied.

Another common complications of AKI in critically ill it is represented by acidosis

Acidosis it's one of the most commonly seen problems in critically ill patients. Respiratory failure, kidney failure, heart failure, Hypoperfusion and intoxications can all result in dangerous shifts of pH concentration. These disturbances often go unnoticed, especially in the case of combined acid-base disorders. If the perturbations are not recognized and addressed, they may result in poor outcomes in intensive care unit (ICU) patients. Acidosis is one of the most common indications for RRT in ICU. While common CRRT buffers can provide restauration of internal milieu, with better control of PH and HCO₃, mechanical ventilation (MV) helps control PaCO₂, PaO₂ and O₂ saturation. The best approach for acid-base analysis is debated[44].

The mechanisms responsible for acid-base balance disorders are not completely understood and we can find controversy in many clinical studies and in medical literature as to what methods should be used.

there are three major methods for describing acid-base disorders and each differs in the way to assess the metabolic component of the acid-base equilibrium.

Classical approaches use Henderson Hasselbach variables with measured and derived parameters and Anion gap (AG) calculation. Siggard-Andersen approach includes the calculation of Base Excess (BE)

and Standard BE (SBE).

Physical-chemical approach apply Stewart's method: each one of these methods has several limitations in the ICU setting

Stewart's approach applies three independent variables to establish the value of pH^+ :

- *Strong Ion Difference (SID)* that is the difference between the sums of concentrations of the strong
- *Carbon dioxide partial pressure (PaCO_2)* that reflect changes in respiratory side of acid-base balance
- *Total Weak Acid [Atot]* that is the total plasma concentration of weak non-volatile acids, inorganic acids [inorganic phosphate, serum proteins, and albumin]
-

For critically ill patients the Stewart approach may be better than the Classic approach, popularized by

Relman and Schwartz, which uses the Bronstead-Lowry definitions.

This approach seems to be more sensitive than classical approach for detection of metabolic acidosis in critical ill patients on CVVH [44].

Certain studies suggest that traditional analysis often fails to identify metabolic acidosis in critically ill patients; Stewart's method allows quantifying the component of acid-base disorders individually and thus offer a better understanding of pathogenesis.

The control of acid base and water homeostasis can be explained in terms of both sodium and chloride regulation: these electrolytes can be manipulated in the clinical setting to optimize therapeutical approach of acid base disorders [45].

We encourage to the use of Stewart's approach for the detection of acid-base disturbances in ICU as well as BE and AG.

Future studies must to be performed to evaluate if an early application of renal replacement therapies in AKI or a larger dose of replacement fluids, such as HVHF or new replacement fluids may improve to the management of acidosis in ICU patients.

Acute Kidney Injury (AKI) in the Intensive Care Unit (ICU) has been shown to be a significant risk factor for mortality, particularly when patients need renal replacement therapy (RRT).

Current guidelines [46] don't recommend extracorporeal treatments in patients with sepsis without renal indications. Nevertheless, the early use of extracorporeal blood purification techniques combined with standard therapies in non-ARF septic patients may modify clinical outcome and improve survival [47]. Extracorporeal blood purification (EBP) is primarily used in

patients with renal failure. More than 20 years ago, it was suggested that EBP could remove inflammatory mediators from the plasma of septic patients and improve pulmonary function. Subsequently, surrogate clinical improvements through cytokine removal from the circulation with hemofiltration were reported in animal and human studies [48]. Most immune mediators are water-soluble and fall into the “middle-molecular-weight” category (5–50 kDa) and hence can be removed by EBP.

Theoretically speaking the perfect treatment for septic shock has to restore hemodynamic stability, remove mediators and re-establish immune response to the host [49].

In our study High Volume Hemofiltration (HVHF) and Coupled Plasma Filtration Adsorption (CPFA) have shown potential improvements in septic animals, but actually there are no studies that compare these two types of treatments in human.

HVHF is a particular hemofiltration with a large use of replacement fluid (more than 50 ml/kg/h); there have been no adverse events in clinical trials for more than 80 hours of therapy. Recently it has been demonstrated in different conditions that it significantly improves patients hemodynamic conditions and modifies cytokines and complement factors plasmatic concentrations in patients with severe sepsis and septic shock. HVHF use reduce the need of vasopressors and significantly modifies endotoxin blood levels compared with “standard” CRRTs. It improves mean arterial pressure and cardiac output in septic shock induced in dogs and porcine models. During HVHF adsorption is increased due to the effects of an increased hemofiltration rate on transmembrane pressure: the recruitment of larger surfaces on one hand and higher permeable membranes on the other. Therefore, in HVHF adsorption overcomes on convection, a condition which can not sufficiently explain all the hemodynamic benefits described [50].

P(pulse) HVHF (pHVHF) is the combination of a HVHF performed for 6 to 10 hours followed by standard CVVH for the rest of the day. Both modalities are usually performed in pre-dilution, although post-dilution can be added (20-40%).

Coupled plasma filtration adsorption (CPFA) is an extracorporeal therapy that uses a plasma filter to separate plasma from the blood; the separated plasma then passes through a sorbent cartridge for the nonspecific removal of various mediators. The plasma is returned to the blood after purification. This blood can then pass through a hemodialyzer/hemofilter for additional

blood purification by conventional hemodialysis, hemofiltration or hemodiafiltration in patients with AKI [51].

CPFA is able to purify mediators that are passed through the plasma into an adsorbent cartridge containing a styrenic resin. This resin has a large surface area (over 700 m²/ml resin) and a high affinity for many cytokines and mediators; it is a synthetic cross-linked styrenic divinylbenzene resin.

The treatment goal of CPFA is to target the excess of circulating mediators in order to counteract the disproportionate pro and anti-inflammatory reaction which occurs in sepsis [52].

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The role of plasma exchange remains equally controversial; still, the initial findings with CPFA are consistent and the early observations are encouraging [53].

Our study suggest that HVHF appears more useful in depuration, reduction of vasopressors requirement and seems to be more useful to improve PaO₂/FiO₂ ratio: on the other hand CPFA reduce Vasopressor Score and AOPP concentration: ideally they appear to be complementary in ICU septic patients [54].

CONCLUSIONS

Patients with Sepsis and AKI showed elevated biomarkers levels (NGAL, BNP, AOPP) compared to ICU patients, particularly in AKI septic patients.

Fluid Overload Management can be effectively improved with non-invasive “static” measurements such as BNP and BIVA as well as minimally invasive “dynamic” measurement as Stroke Volume Variations: they can be safely used at bedside to add additional information to the clinical standard monitoring.

Our results suggest that Stewart approach can be associated to Classic approaches to improve buffer administration or optimize extracorporeal treatments.

In the same directions we need emerging treatments to improve RRT in AKI-Septic patients, able not only to replace renal function but also to ensure Multi Organ Support Therapies.

New Technologies, New Biochemical parameters and new treatments can improve early diagnosis, monitoring and treatment in AKI septic and Non-Septic patients.

I hope that these preliminary results will encourage further study of these important questions.

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