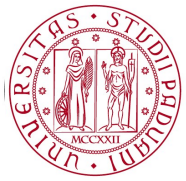

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Dipartimento di Nefrologia

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CICLO XXIV°

Sepsis and AKI in ICU Patients: the role of Plasma Biomarkers

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SOMMARIO

La sepsi è una delle cause principali di morbidità e mortalità nei pazienti critici in Terapia Intensiva. I batteri Gram-negativi sono implicati nel 50-60% dei casi di sepsi in Terapia Intensiva e l'endotossina svolge un ruolo importante nella patogenesi dello shock settico.

La sepsi è anche un fattore che contribuisce in oltre il 20% dei casi al danno renale acuto nei pazienti in terapia intensiva, e in alcuni casi vi è la necessità di una terapia renale sostitutiva. L'insufficienza renale acuta (IRA) si verifica nel 35-65% dei ricoveri in Terapia Intensiva e la maggior parte degli studi mostrano un aumento di cinque volte sul rischio di morte tra i pazienti con danno renale acuto rispetto ai pazienti senza IRA.

Dato il tasso di mortalità più elevato di pazienti in Terapia Intensiva con sepsi e IRA, si è indagato nei pazienti settici della Terapia Intensiva sulla possibile correlazione tra i biomarcatori di danno d'organo (NGAL, AOPP e BNP) e l'attività dell'endotossina. Inoltre, il confronto dei livelli di questi marcatori sono stati analizzati tra i pazienti settici e non settici, pazienti settici con o senza IRA e tra i pazienti che hanno sviluppato danno renale acuto, con o senza sepsi.

Novantotto pazienti adulti ricoverati in Terapia Intensiva dell'Ospedale di San Bortolo, Vicenza, Italia, tra ottobre 2008 e agosto 2010, sono stati arruolati in questo studio. I pazienti sono stati divisi in due gruppi a seconda della presenza di sepsi, definita come Sindrome da Risposta Infiammatoria Sistemica (SIRS) associata a un processo infettivo. Cinquantasei pazienti presentavano sepsi, mentre 42 pazienti non presentavano sepsi. Tra i pazienti settici, 24 soggetti hanno sviluppato IRA, definita dai criteri RIFLE, mentre 32 non hanno IRA. Una correlazione significativa tra l'attività di endotossina e i biomarcatori è stata trovata solo con i livelli di BNP dei pazienti settici ($p=0,02$). I livelli di NGAL, BNP e AOPP erano significativamente più alti tra i pazienti settici rispetto ai soggetti non settici ($p<0,001$). Tra i pazienti settici, i soggetti che hanno sviluppato IRA hanno mostrato livelli più alti di NGAL e AOPP ($p=0,0425$), e BNP ($p=0,0327$).

La correlazione tra l'attività dell'endotossina e i livelli di BNP nei pazienti settici, e l'aumento dei livelli di NGAL, BNP e AOPP in caso di sepsi e IRA, in particolare se

sono associati, indicano un coinvolgimento multiorgano in queste condizioni. La loro valutazione può permettere ai medici di individuare prima i pazienti a maggior rischio di morbidità e mortalità.

ABSTRACT

Sepsis is a primary cause of morbidity and mortality in intensive care unit (ICU) and critically ill patients. Gram-negative bacteria are implicated in 50-60% of cases of sepsis in ICU and endotoxin is considered to play an important role in the pathogenesis of septic shock.

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.

Given the higher mortality rate of ICU patients with sepsis and AKI, we decided to 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) and endotoxin activity in ICU septic patients. Moreover, comparisons of the levels of these biomarkers were made between septic and non septic patients, septic patients with or without AKI and between patients who developed AKI with or without sepsis.

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.

A significant correlation ($p=0.02$) was found only between endotoxin activity and BNP levels of septic 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 and AOPP ($p=0.0425$), and BNP ($p=0.0327$). Among patients

who developed AKI, a significant difference was found only in terms of AOPP levels between septic and non septic patients.

The correlation between endotoxin activity and BNP in septic patients and the increase in the levels of NGAL, BNP and AOPP in case of sepsis and AKI, in particular if they are associated, indicate a multiorgan involvement in these two conditions. Their evaluation can allow clinicians to individualize earlier patients at higher risk of morbidity and mortality.

INTRODUCTION

Sepsis

Sepsis is a serious clinical condition, characterized by the presence of acute inflammation which involves the whole body, and is associated with the presence of a known or suspected infection [1-2].

According to the modern concept of sepsis, the host's immune response to the infection is responsible for the most part of the symptoms, hemodynamic changes and organ damage. This host response has been defined "Systemic Inflammatory Response Syndrome" (SIRS) and is characterized by hemodynamic alterations and consequent metabolic derangement.

Sepsis and SIRS are common and represent a major cause of morbidity and mortality in Intensive Care Unit (ICU) and the critically ill patients.

The pathogenesis of these syndromes has been widely studied and the clinical outcome of these clinical conditions is improving.

In 1991, the American College of Chest Physicians and the American Society of Critical Care Medicine published the definitions of SIRS and Sepsis to clarify the diagnosis and the treatment of these conditions and to help researchers in their interpretation [3].

There are different stages of sepsis [Table 1].

Table 1: Definitions for SIRS and Sepsis

SIRS	Defined by the presence of two or more of the findings	* Body Temperature <36°C or >38°C * Heart Rate >90 beats per minute * Respiratory Rate >20 breaths per minute or a $PaCO_2$ <32mm Hg * WBC <4,000 cells/mm^3 or >12,000 cells/mm^3
Sepsis	Defined as SIRS in response to a confirmed infectious process	Infection can be suspected/proved by * Culture * Stain * PCR or a clinical syndrome pathognomonic for infection
Severe Sepsis	Defined as sepsis with organ dysfunction, hypoperfusion, or hypotension	
Septic Shock	Defined as sepsis with refractory arterial hypotension or hypoperfusion either end-organ dysfunction or serum lactate greater than 4 mmol/dL	End-organ dysfunction include [4] * Lungs * Brain * Liver * Kidney * Heart

However, consensus definitions continue to evolve and the latest ones have extended the list of signs and symptoms of sepsis to reflect clinical bedside experience [1].

Sepsis requires a significant consumption of intensive care resources and remains an ever-present problem in the ICU [3]. It is common and also more dangerous in elderly, immunocompromised, and critically ill patients [5].

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 one of several ICU scoring systems and it is used to track a patient's status during the stay in an ICU [6]. The score is based on six different scores for respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems.

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 [7], with mortality rates that range from 20% for sepsis to 40% for severe sepsis to 60% for septic shock [8].

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 [9].

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 result from poorly controlled infections, immuno-suppression, complications of intensive care, failure of multiple organs, or patient's underlying diseases [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 is due to the less common causes of fungi, viruses and protozoa [3].

The development of Gram-negative sepsis involves complicated series of effects based on the composition of the bacterial cell wall.

Pfeiffer first recognized the heat-stable toxic component of Gram-negative bacteria at the end of the 19th century [11-12]. He called the toxic substance, not yet characterized, "endotoxin" on the assumption that it was found inside the bacterium. In the 1930*s*, endotoxins were isolated and characterized as lipopolysaccharide (LPS)-phospholipid-protein complexes present in the bacterial outer membrane [13-14].

Endotoxin is one of the principal bacterial components that exist in the outer membrane of Gram-negative bacteria and interact with the host during Gram-negative bacterial sepsis. It causes the release of different cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), and activates complement and coagulation factors [15-16]. Therefore, endotoxin is considered one of principal biological substances that cause Gram-negative septic shock [17].

Endotoxin Activity Assay

A new and reliable Endotoxin Activity Assay (EAA^{tm}) has been recently validated in clinical practice [18]. Briefly EAA^{tm} is based upon the degree of priming of the circulating neutrophil population by endotoxin exposure.

Endotoxin activity levels are expressed as units on a scale ranging from 0 to 1: low (<0.4 units), intermediate (0.4<X<0.6 units) and high (>0.6 units).

In a recent trial, Marshall et al. [18] reported that endotoxemia is common in a heterogeneous population of critically-ill patients on the day on admission in ICU: more than 50% of all patients have intermediate or high levels of endotoxin activity as compared to healthy volunteers.

In a recent study of Monti et al., high levels of endotoxin activity in septic shock patients were found to correlate with the severity of the disease, and in particular with hemodynamic dysfunction [19].

AKI

Acute Kidney Injury (AKI) is characterized by the rapid loss of kidney function. AKI is common among hospitalized patients. It affects 3-7% of patients admitted to hospital and approximately 25-30% of patients in the ICU [20].

The aetiology of AKI in critically ill patients is often multifactorial. However, sepsis has been found to be a leading contributing factor to AKI in critical ill patients [21-28]. Discriminating between AKI of septic and no-septic AKI may have clinical relevance [29]. Evolving data suggest that septic AKI may be characterized by a distinct pathophysiology [30-33].

Septic AKI occurs in 15-20% 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 septic AKI pathogenesis still remains poorly understood [34].

The diagnosis of AKI is generally made using urine output and serum creatinine.

The use of urine output and serum creatinine has dominated the clinical scenario for many years, and nowadays it is important in the diagnosis of AKI. They represent, indeed, two criteria necessary for the diagnosis of AKI in RIFLE classification system [35-36].

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, given the growing interest of researchers over the last few decades in this field [37]. The RIFLE criteria classify renal dysfunction according to the degree of the renal impairment: there are three stages of severity (Risk, Injury, and Failure), and two outcome classes (Loss of kidney function, and End-stage kidney disease) [Figure 1].

The RIFLE classification has been evaluated and validated in numerous clinical studies carried out in critically ill patients, post-operative and burned patients, and it was found to be a valid tool for the early diagnosis and staging of AKI, having a good predictive value for mortality [38-43].

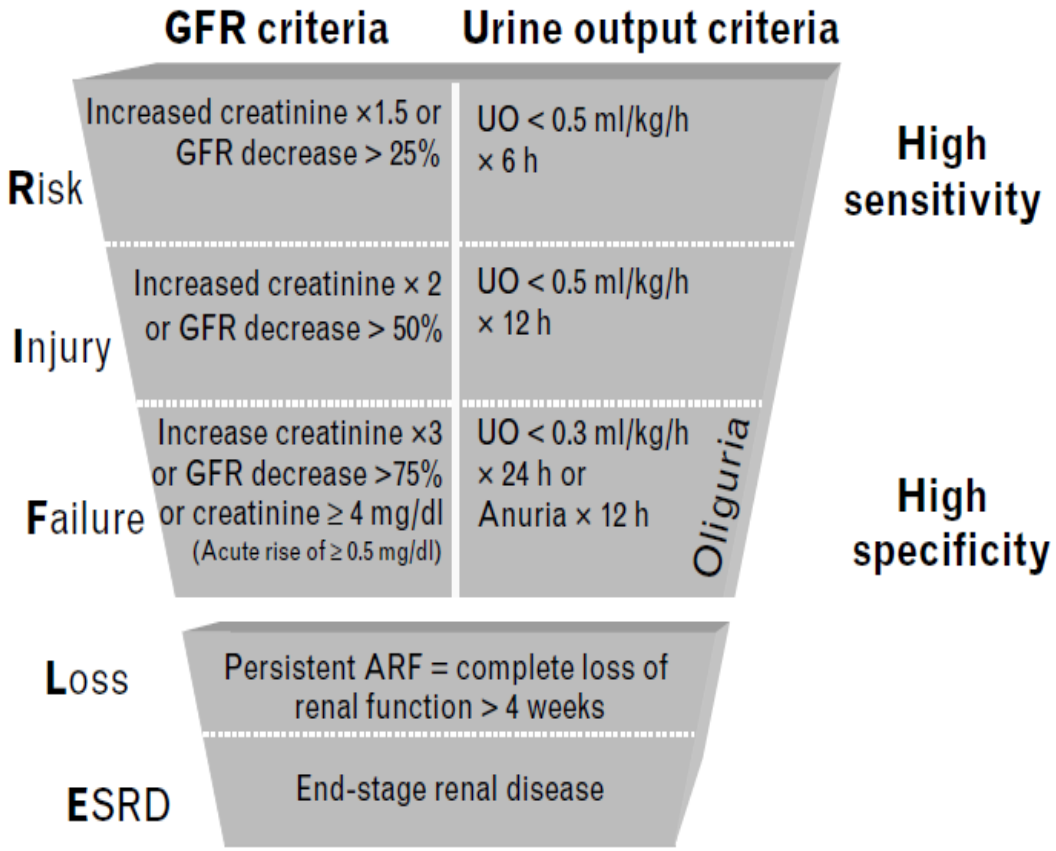
The diagnosis and etiological classification of AKI, at present, largely depend on the detection of changes in conventional endogenous surrogate markers of kidney

function, especially serum levels of creatinine and urea. These tests are familiar to clinicians and have been used at the bedside for a long time. Unfortunately, however, these markers are not ideal, because they have some limitations and none reflects real-time dynamic changes in Glomerular Filtration Rate (GFR) or genuine kidney injury [39].

Novel biomarkers beyond urine output and creatinine can help in an easier and earlier diagnosis of AKI. 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 [44].

Figure 1: 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.



NGAL

Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) was originally identified in neutrophils as a 25-kDa protein covalently bound to gelatinase.

NGAL is normally expressed at very low levels in several human tissues, including kidney, lungs, stomach, and colon. NGAL expression is markedly increased in injured epithelia, and in the serum of patients with acute bacterial infections [45]. NGAL seems to be one of the earliest markers of ischemic or nephrotoxic kidney injury in animal models, and it is detected in the blood and urine of humans soon after AKI [46-50]. Several studies have confirmed these findings. In ICU patients with AKI secondary to sepsis, ischemia, or nephrotoxins, NGAL is significantly increased in plasma and urine when compared to normal controls [51].

A recent systematic review and meta-analysis of NGAL studies using standardized data sheets sent to authors, confirmed the value of NGAL as an early predictor of AKI across settings. Urine and plasma NGAL performed similarly well. NGAL level had prognostic value for renal replacement therapy and mortality [52]. Its concentration increases dramatically in response to tubular injury and precedes rises in serum creatinine by >24 hours [53].

Urine and plasma NGAL is now the most promising novel renal biomarker [54-56]. As a general rule, a concentration >150 ng/mL can identify patients at high risk for AKI, and a level >350 ng/mL those at high risk for renal replacement therapy [57].

It should be considered that NGAL levels may be influenced by coexisting factors, such as pre-existing renal disease [58] and systemic or urinary tract infections [59].

Oxidative Stress

In sepsis, an overwhelming inflammatory response results in excessive production of free radicals. The action of these molecules is normally limited by antioxidant systems. However, in septic patients the antioxidant capacity is likely to be compromised [60]. Evidence of elevated oxidative stress (OS) is well established in critical illnesses, namely in sepsis [61-62]. Moreover, a discriminative power to predict outcome was also found in some previous studies [63].

Advanced oxidation protein products (AOPP) have been found to be a simple technique for monitoring OS, especially in critically ill patients [64].

In several studies the presence of AOPP was shown to be not only an important marker of OS, but also an inflammatory mediator with an important role in the pathogenesis of acute diseases and chronic renal failure [65].

CRS

A large proportion of patients admitted to hospital, especially in the critical care setting, have various degree of heart and kidney dysfunction [66]. Primary disorders of one of these two organs often result in secondary dysfunction or injury of the other one [67].

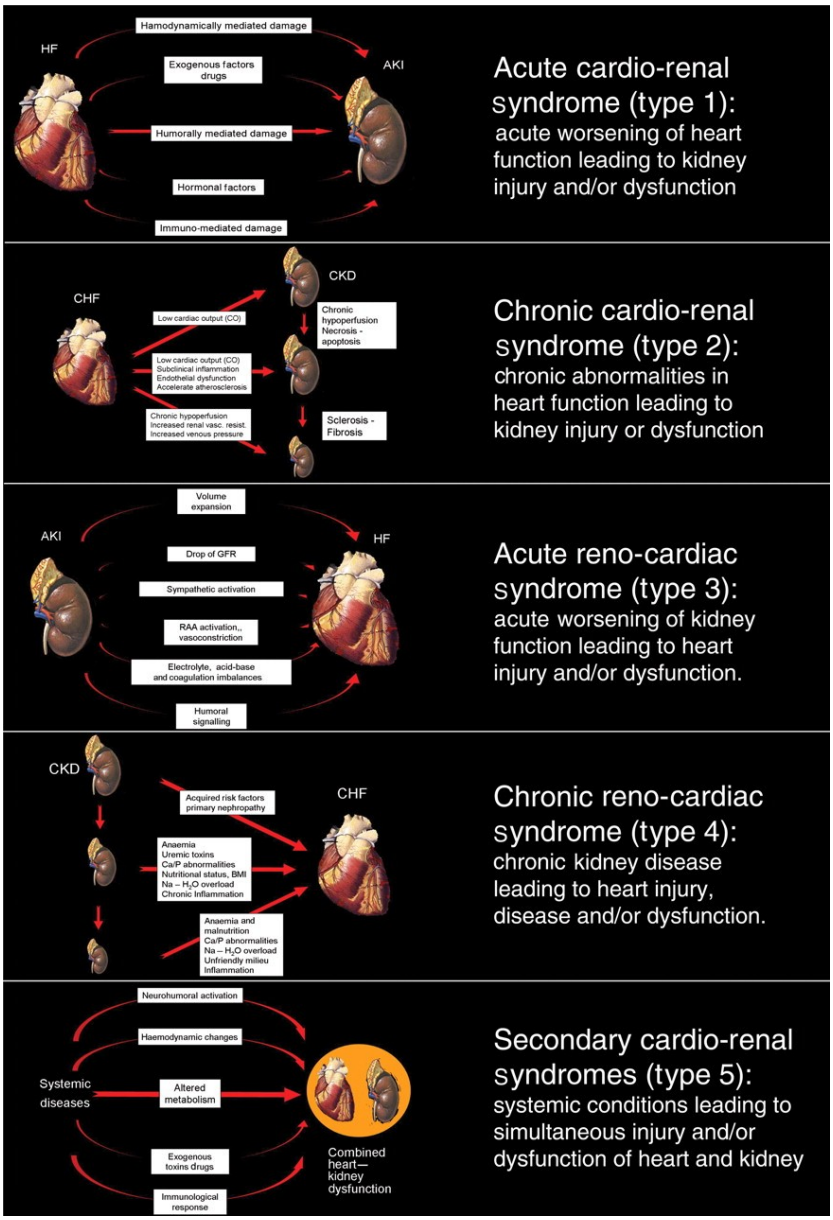
According to the general definition of Cardio-Renal Syndrome (CRS), it is a disorder of the heart and kidney whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other one. Five subtypes have been defined, reflecting the primacy of organ dysfunction and the time-frame of the syndrome [68].

Different pathophysiological 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 [69]. Actually, sepsis represents a prototypal condition that may cause an acute form of Type 5 CRS. Approximately 11-64% of septic patients develop AKI [70], and 46-58% have sepsis as a major contributing factor to development of AKI [71]. Similarly, abnormalities of cardiac function are common in septic patients [72].

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 [73].

Figure 2: Pathophysiology and Definitions of the Five Subtypes of Cardio-Renal Syndrome



BNP

Brain Natriuretic Peptide (BNP) is a neurohormone secreted from ventricular myocardium in response to myocardial stretching and volume overload [74].

BNP has diagnostic and prognostic utility in patients with acute decompensated heart failure [75-78], and it is an independent predictor for cardiovascular events and overall mortality in various patients groups, including those with chronic kidney disease [79-82].

Burchill et al. have shown, in experimental animal models, that the acute effects of AKI on the heart occur as early as few hours after kidney injury and that changes in cardiac structure are associated with increased cardiac BNP [83].

Moreover, in my recent study I showed that critically ill patients with AKI have high levels of BNP compared to No-AKI patients [84].

Myocardial dysfunction is a common complication in patients with severe sepsis [85]. BNP concentration represents a reliable marker for identification of patients developing sepsis-induced myocardial depression [86].

AIM OF THE STUDY

The present study aimed to:

1- Analyse the biochemical markers in septic patients' population.
Plasma NGAL for kidney injury, AOPP for OS and plasma BNP for heart failure were evaluated

2- Analyse possible correlations between EAA levels and the biochemical markers

3- Compare biochemical markers in AKI and No-AKI septic patients to ICU patients

4- Evaluated CRS markers in the septic patients' population

METHODS

Fifty-six adult patients admitted to the ICU of San Bortolo Hospital, Vicenza, Italy, were recruited for this study. SIRS was considered to be present when at least two of the following criteria were present: temperature above 38°C or below 36°C, heart rate above 90 beats/min, respiratory rate above 20 breaths/min or partial pressure of carbon dioxide below 32 mmHg, white blood cell count above 12,000 mm^3 or below 4,000 mm^3. Sepsis was defined as SIRS associated with an infectious process.

AKI was defined using the creatinine and urine output criteria of the RIFLE classification. Twelve patients (23%) had AKI at the moment of recruitment for the study.

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

Endotoxin Activity Assay (EAA)

The EAA^{tm} is a rapid testing device, which measures the serum endotoxin activity. This test allows the measurement of endotoxin activity as a function of each patient's neutrophil chemiluminescence's activity (on a scale from 0 to 1).

The test is based on the reaction of endotoxin with a specific anti-endotoxin antibody. Complement proteins opsonize the endotoxin-antibody complex. The opsonized immune complex primes neutrophils in the blood to enhance their respiratory burst in response to zymosan. The respiratory burst of the neutrophils yields oxidants that react with luminal in the reaction mixture to emit chemiluminescence.

The chemiluminescence can then be detected in a photon counting luminometer (SmartLine TL, Berthold Detection Systems, Pforzheim, Germany) [Figure 3].

A basal activity measurement (Tube1) in the absence of the specific anti-endotoxin antibody measures the non-specific oxidative burst of the patient's neutrophils. An additional control measurement including the specific anti-endotoxin antibody and an excess of exogenous endotoxin (Tube3) measures the maximum oxidative burst of

the patient's neutrophils. The test measurement (Tube2) includes the specific antibody to measure the neat level of endotoxin activity. The EAA^{tm} level is calculated by normalizing the chemiluminescence in the test sample (Tube2) against the maximum chemiluminescence (Tube3), correcting both measurements for the basal activity chemiluminescence (Tube1).

Interpretation of Results:

0.00 – 0.39 EAA^{tm} units
low endotoxin activity level represents

* rule-out the presence of a Gram- bacterial infection
* a low risk for progression to severe sepsis

0.40 – 0.59 EAA^{tm} units
intermediate endotoxin activity level represents an elevated risk for severe sepsis

≥ 0.60 EAA^{tm} units
high endotoxin activity level represents a high risk for developing severe sepsis

NGAL and BNP Measurement

Plasma samples for NGAL and BNP measurement were quickly stored at minus 80 degrees Celsius to be analyzed subsequently. Plasma NGAL and BNP were measured with fluorescence-based immunoassay with the Triage point-of-care analyzer (Biosite Inc., San Diego, CA, USA) [Figure 4], which allows a rapid quantitative measurement of NGAL and BNP concentration in EDTA-anticoagulated whole blood or plasma.

AOPP Measurement

AOPP were measured by spectrophotometry and calibrated with Chloramine-T solutions (Sigma Chemical Co., St. Louis, MO, USA), which adsorb at 340nm in

presence of potassium iodide. Two hundred microliters of plasma diluted 1/5 in PBS and 20µl 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 10µl of potassium iodide.

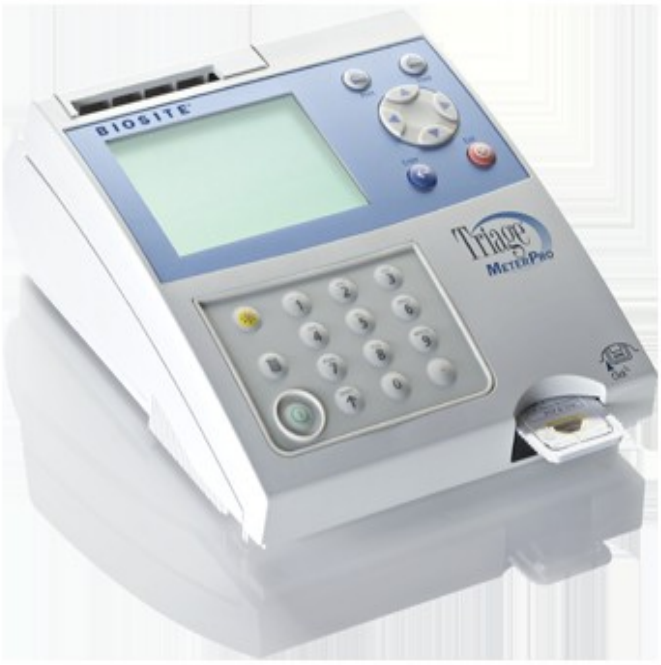
The absorbance of the reaction mixture was read at 340nm against a blank containing 200µl of PBS, 10µl of potassium iodide, and 20µl of acetic acid. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

Statistical Analysis

Statistical analysis was performed with the use of SPSS software version 15.0. Categorical variables were expressed as percentages; continuous variables were expressed as means ± standard deviation (parametric variables) or median (interquartile range; non parametric variable). Differences between groups were analyzed using Student t-test and Mann-Whitney test as appropriate. Correlation was performed with the use of the Spearman rank coefficient. Two-tailed probability values of $<.05$ were considered statistically significant.

Figure 3: SmartLine TL, Luminometer for endotoxin activity detection

Figure 4: Triage point-of-care analyzer, for NGAL and BNP evaluation


BIOSITE
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RESULTS

Sixty-four consecutive adult patients were enrolled in San Bortolo Hospital ICU. Eight patients were excluded for uninterpretable results, leaving 56 patients for analysis [Table 2].

Table 2: Clinical and biochemical characteristics of septic patients

	ALL PATIENTS (N=56)
Male sex (%)	33.9
Age (years)	69 (48.7 to 74.2)
Serum creatinine (mg/dL)	1.64 (1.04 to 2.97)
Urea (mg/dL)	99.04 ± 103.71
Temperature (°Celsius)	36.4 ± 2.0
WBC (million cells/mcL)	12.3 ± 9.4
Platelets (10^3/μL)	144.6 ± 110.9
pH	7.359 ± 0.154
Na (mmol/L)	139.5 ± 6.1
K (mmol/L)	4.2 ± 1.1
PaO_2/FiO_2 (mmHg)	206.4 ± 112.5
EAA (Units)	0.68 ± 0.28
NGAL (ng/mL)	459 (213 to 744)
AOPP (μmol/L)	505.1 (307.6 to 643.5)
BNP (pg/mL)	409 (212 to 673)
SOFA Score	10 (8 to 12)
Died (%)	32.1

WBC: white blood cells; SOFA score: Sequential Organ Failure Assessment

Data showed correlations between creatinine and NGAL (p<0.0001) [Figure 5] and NGAL and SOFA score (p=0.009). These data confirmed previous findings related to correlation between creatinine and NGAL.

Endotoxin activity levels were expressed as units on a scale ranging from 0 to 1: low (<0.4 units), intermediate (0.4<X<0.6 units) and high (>0.6 units).

Septic patients were divided into three groups depending on measured endotoxin levels. There was a correlation between EAA levels measure and biomarkers because patients with low risk for progression to severe sepsis showed low levels of NGAL, BNP and AOPP. [Figure 6a,b,c] Only for BNP there was a significant p value.

Figure 5: Correlation between NGAL and creatinine in 56 septic patients (p<0.0001)

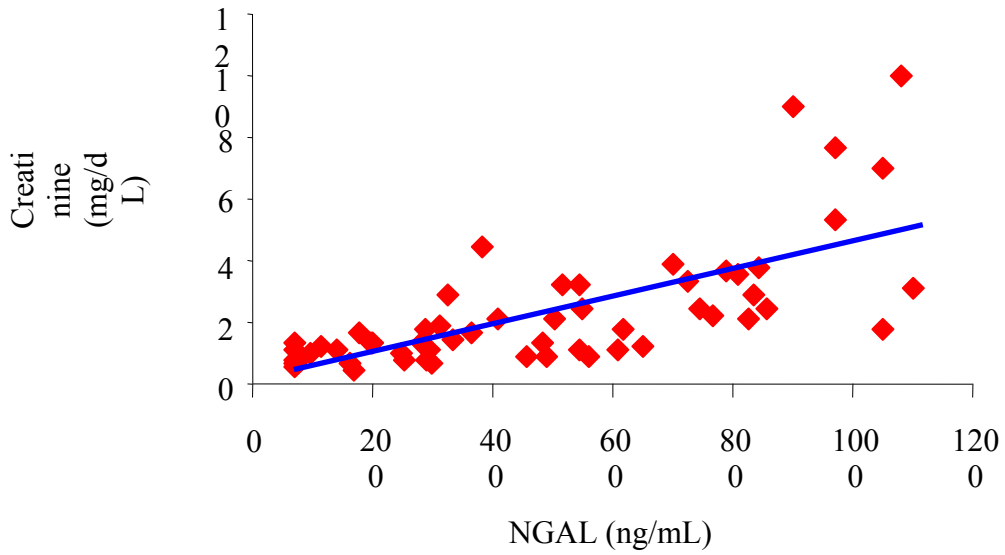


Figure 6: Correlations between EAA levels (<0.40; 0.40-0.60; >0.60 units) and the biochemical markers (NGAL:6a; BNP:6b; AOPP:6c)

Figure 6a: NGAL

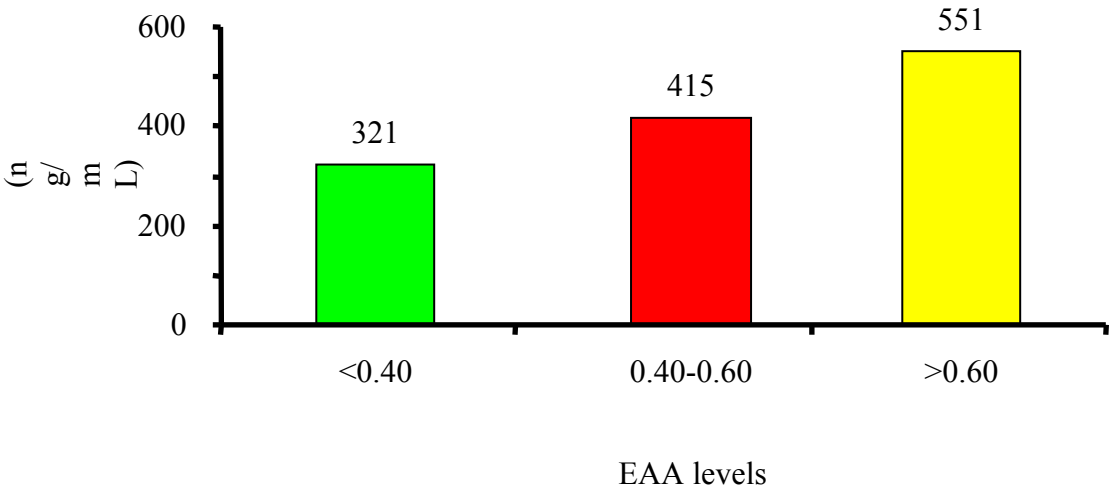


Figure 6b: BNP p=0.02

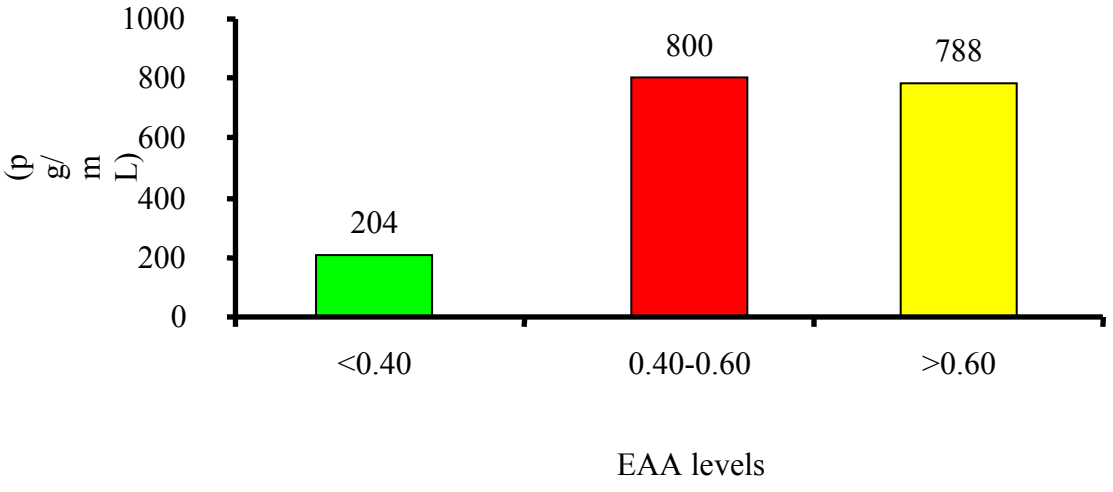
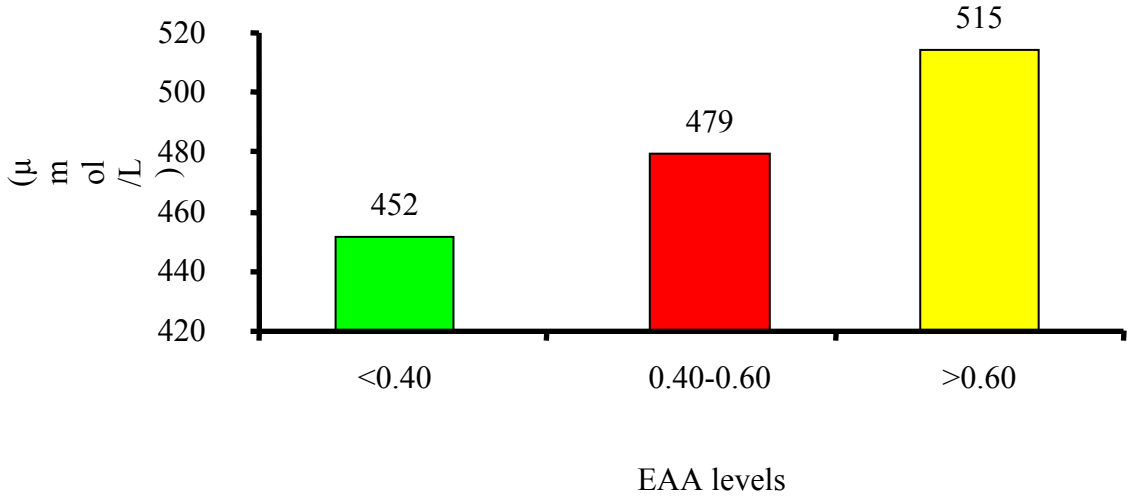


Figure 6c: AOPP



Septic patients were compared to an ICU population (42 patients).

The levels of creatinine, NGAL, BNP and AOPP were significantly higher among septic patients compared to the ICU population. [Table 3]

Table 3: Biochemical markers in septic patients and in ICU patients

	SEPTIC PTS (N=56)	ICU PTS (N=42)	*p* value
Male sex (%)	33.9	66.7	0.0013
Age (years)	69 (48 to 74)	67 (59 to 75)	0.83
Creatinine (mg/dL)	1.6 (1.0 to 3.0)	1.0 (0.8 to 1.0)	<0.001
NGAL (ng/mL)	459 (213 to 744)	120 (79 to 174)	<0.001
AOPP (µmol/L)	505.1 (307.6 to 643.5)	115.7 (79.2 to 181.7)	<0.001
BNP (pg/mL)	409 (212 to 682)	135 (61 to 275)	<0.001
SOFA Score	10 (8 to 12)	5 (4 to 5)	<0.001
Died (%)	32.1	16.7	0.08

Therefore, all biomarkers levels differed significantly between septic and ICU patients. The two groups of patients were divided according to the presence or the absence of AKI to better understand what was the cause of the difference between the levels of the biomarkers.

In septic patients' population, 24 patients (42.9%) had AKI upon admission. Creatinine, NGAL, BNP and AOPP were significantly higher among AKI septic patients compared to No-AKI septic patients. [Table 4]

Table 4: Biochemical markers in AKI and No-AKI septic patients

	AKI SEPTIC PTS (N=24)	NO-AKI SEPTIC PTS (N=32)	*p* value
Male sex (%)	29.2	37.5	0.51
Age (years)	69 (50 to 71)	69 (45 to 76)	0.63
Creatinine (mg/dL)	2.3 (1.5 to 3.4)	1.2 (0.8 to 1.9)	0.0065
NGAL (ng/mL)	572 (308 to 819)	321 (154 to 573)	0.0425
AOPP (µmol/L)	554.0 (366.8 to 717.6)	419.5 (286.8 to 607.4)	0.0425
BNP (pg/mL)	576 (291 to 1723)	348 (174 to 538)	0.0327
SOFA Score	11 (8 to 13)	9 (7 to 12)	0.28
Died (%)	45.8	21.9	0.0575

Moreover, data from AKI septic patients were compared (24) with data from AKI ICU patients (14) and significant differences in creatinine and AOPP levels were found while no differences in NGAL and BNP levels were observed. [Table 5]

Table 5: Biochemical markers in AKI septic patients compared with AKI ICU patients

	AKI SEPTIC PTS (N=24)	AKI ICU PTS (N=14)	*p* value
Male sex (%)	29.2	78.6	0.0033
Age (years)	69 (50 to 71)	76 (69 to 80)	0.0067
Creatinine (mg/dL)	2.3 (1.5 to 3.4)	1.0 (1.0 to 1.0)	<0.001
NGAL (ng/mL)	572 (308 to 819)	312 (141 to 633)	0.15
AOPP (µmol/L)	554.0 (366.8 to 717.6)	118.9 (90.1 to 152.5)	<0.001
BNP (pg/mL)	576 (291 to 1723)	305 (134 to 559)	0.1055
SOFA Score	11 (8 to 13)	5 (5 to 5)	<0.001
Died (%)	45.8	42.9	0.85

DISCUSSION

This study is aimed to examine whether a correlation between endotoxin levels and cardio-renal biochemical markers exists and to compare biochemical markers in AKI and No-AKI septic patients to ICU patients.

Biomarkers are biological substances produced in human physiological processes which reflect functional changes or organ injury. The ideal biomarker is easily measured, specific for the organ under investigation, soon released after injury, and related to the degree of injury and prognosis [87].

Sepsis is one of the most common causes of death in ICU patients [88] and mortality is even higher when AKI develops [89].

The last decade has witnessed many advances in the treatment of AKI. However, morbidity and mortality have not improved significantly [90-92]. Serum creatinine is an insensitive and late marker [93] of AKI. Since it is important to diagnose AKI as early as possible to facilitate effective interventions [94], an aggressive search for newer biomarkers has been carried out.

NGAL has recently 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 has been shown to possess various biological properties, for instance kidney-protection and nephron-inducing activity and bacterio-static capability [95]. Although normally expressed at very low levels in different tissues, 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 [96]. Furthermore, the rise of NGAL is observed before plasma creatinine increases, allowing an early detection of AKI with good sensitivity and excellent specificity [97]. 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 [98]. The acute rise of NGAL in most reported studies developed 2-6 hours after kidney injury [95].

NGAL has been shown to be elevated in patients with Congestive Heart Failure (CHF), possibly demonstrating a link between cardiac dysfunction and renal injury [99-100].

In this study data showed that lower endotoxin levels correlated with lower NGAL levels and these values increased with the risk of developing severe sepsis.

According to previous studies, NGAL levels related to creatinine, and they were significantly higher in septic patients compared to ICU patents. Moreover, 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 in NGAL concentration and this may be due to infectious processes rather than renal damage.

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

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

OS has been demonstrated to play a pivotal role 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 factors may contribute to extra-cellular and intra-cellular increase in oxidative 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, and apoptosis). The role and importance of the ROS in the regulation of these functions during critical illness is only partially understood [101].

Several studies have evaluated OS in critical illness. Himmelfarb et al [102] have 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 et al. [101] measured AOPP for 4 days after admission in ICU patients and therefore they were able to appreciate the fluctuation of OS over time. This was the first study to assess the correlation between OS and the severity of AKI. By showing that patients with AKI have significantly elevated AOPP levels, as compared with No-AKI patients, Lentini et al. confirmed previous findings related to elevated OS in AKI [102-104], 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 class Risk and Injury AKI patients.

It also remains unclear whether elevated OS is a simple epiphenomenon, or it is causative for AKI [101].

In this study AOPP levels reflected the severity of sepsis. A low risk of severe sepsis correlated to lower levels of AOPP, and data showed a not significant increase with the higher risk for developed severe sepsis.

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

BNP have gained success as a diagnostic and prognostic biomarker, especially among CHF patients [105]. 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 [106]. In this study, the levels of BNP were significantly higher in septic patients compared to ICU patients, and moreover in AKI septic patients compared to No-AKI septic patients.

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 the assessment of CRS [69,107].

Burchill et al. have shown in experimental animal models that the acute effects of AKI on the heart occur as early as few hours after kidney injury and that changes in cardiac structure are associated with increased cardiac BNP [83].

CONCLUSIONS

In septic ICU patients endotoxin activity correlates with BNP levels. NGAL, AOPP and BNP levels seem to be higher in patients with sepsis and AKI, in particular if they are associated. In case of AKI, a significant difference between septic and non septic patients was found only for AOPP levels.

NGAL, AOPP and BNP increase in case of sepsis, thus indicating both cardiac and renal impairment (Cardio-Renal Syndrome).

For this reason, the rise in their levels in this condition can allow clinicians to individualize patients at higher risk for developing severe sepsis, and therefore at higher risk of death.

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