

## Role of Angiotensin-2 in Medical-Ward Patients with SIRS/Sepsis

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**Abstract:** *Background:* SIRS/sepsis accounts for vast majority of deaths in ICU a medical ward department. More than 650,000 cases of sepsis are diagnosed in the United States annually, with 30-50% mortality and individually cost more than 22,000 dollars.

Biomarkers could be useful tools for early risk stratification in these patients. Angiotensin-2 (Ang-2) is a proinflammatory mediator of endothelial injury, which has received considerable attention over the past decade but little is known about its correlation with organ failure and mortality in SIRS/sepsis admitted to a medical ward.

*Methods:* Ang-2 plasma levels, Charlson index, SOFA and routine laboratory test were carried out in 80 SIRS patients admitted to medical ward within 4 hours from diagnosis.

Survival and organ dysfunction in the following week were recorded.

Investigators were blinded from Ang-2 results.

*Results:* Ang-2 plasma levels were higher in patients suffering from renal, pulmonary and hemostatic dysfunction (16, 6, 4% respectively) and patients who died within 1 week (11%). Ang-2 plasma levels higher than 15  $\mu\text{mL}$  account for 13 fold increased risk of death with 94% negative predictive value.

*Conclusions:* Ang-2 plasma level at admission is predictive of early mortality and kidney, lung and hemostasis dysfunction in SIRS/sepsis patients newly admitted in a medical ward.

**Keywords:** Angiotensin-2, SIRS, sepsis, medical ward, biomarkers.

### INTRODUCTION

Sepsis is a complex syndrome that results from a systemic host's response to infection. Clinically is defined as the presence of infection in conjunction with SIRS (systemic inflammatory response syndrome).

Together, SIRS/sepsis accounts for 650,000 cases in the United States each year [1-3] and are a leading cause of death in ICU, ranging from 30 to 50% especially in the elderly [2], immunosuppressed patients and during multidrug-resistant infections [4]. Beyond early diagnosis, death and organ dysfunction risk stratification of SIRS/sepsis patients is needed to detect those who benefit from a more aggressive approach. Measurement of plasma levels of biomarkers is a valued method for such assessment. Three biomarkers, C-reactive protein [5], procalcitonin [6] and serum lactate [7, 8], have been evaluated in research trials, and many others

are currently assessed, thus reflecting the lack of consensus on the specific prognostic biomarker to use. Angiotensin-2 (Ang-2) is an angiogenic growth factor secreted by activated endothelial cell which acts as an autocrine mediator of the endothelium, increasing vascular inflammation and expression of adhesion molecules [9, 10]. Also, a seminal study identified plasma levels of Ang-2 as a mortality indicator in SIRS/sepsis medical patients [11], but little is known in medical ward, where SIRS criteria are met in around 30% of admissions.

### MATERIALS AND METHODS

Eighty consecutive patients with the diagnosis of SIRS (according to 1991's criteria) [12] and admitted in a medical ward at the Teaching Hospital in Trieste, Italy. Exclusion criteria were age <18 years, prior antibiotic therapy, lack of written consent, and pregnancy. Blood samples for laboratory examination and biomarkers were drawn within 4 h from entering the ward and plasma samples were stored at -20°C within 1 hour from collection. Microbiological sepsis was defined as clinical evidence of sepsis supported by pathogen growth from specimen culture, clinical sepsis was

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**Table 1. Characteristics of the Population. Data are Reported as Median (Interquartile)**

	SIRS	Clinical Sepsis	Microb. Sepsis
N	20	40	20
Age	80.5 (70-83)	83 (75-90)	82 (74-90)
Gender (M/F)	15/5	19/21	10/10
SOFA Score	2 (1-3)	3 (2-4)	3 (2-4)
Charlson Index	2 (0-3)	3 (2-4)	3 (2-5)
Mortality	3	3	3

**Table 2. Ang-2 Levels in Death and Organ Failure. Ang Levels are Reported as Median (Interquartile)**

	Ang-2 ( $\mu\text{g/mL}$ )		Cutoff 15 $\mu\text{g/mL}$	
	Positive	Negative	PPV	NPV
Death (9)	12.4 (8.9-17.1)	5.1 (3.4-9)**	50	94.4
Respiratory (5)	16.6 (16.4-17)	5.2 (3.2-9.2)*	50	98.6
Hemostasis (3)	16.4 (16-45.4)	5.2 (3.3-9.3)*	100	93.5
Liver (6)	4.1 (3.3-16.4)	5.9 (3.4-10.0)	25	94.4
Heart (13)	7.3 (4.4-13.6)	5.1 (3.2-9.7)		
C Nerv Syst. (2)	5.6 (4.8-6.4)	5.6 (3.3-10.2)		
Kidney (13)	15.5 (7.8-17.1)	4.8 (3.1-8.3)*	88	92

\* $P < 0.05$ , \*\* $P < 0.01$ 

defined as clinical evidence of sepsis with negative cultures. Clinical workup and diagnosis have been carried out unaware of biomarkers results. Endpoints of the study were death within 7 days from admission and organ failure. SOFA score and Charlson Index were calculated. Circulating Angiotensin-2 was measured using an ELISA kit developed by R&D Systems Inc. (Minneapolis, MN USA) according to the manufacturer's instructions; the detection limit was 8.29  $\text{pg/mL}$ .

## STATISTICAL ANALYSIS

Mann-Whitney test was used for comparison among groups and Receiver Operator Characteristic (ROC) curve has been carried out for each condition. Based on ROC curves, a discriminating cutoff level of 15  $\mu\text{g/mL}$  was identified for death and other endpoints. Based on  $\chi^2$  test, positive and negative predictive values (PPV and NPV, respectively), and Odds Ratio (OR) for each complication or death were determined. Considering Bonferroni correction for multiple comparisons,  $P$  values below 0.00625 were considered statistically significant.

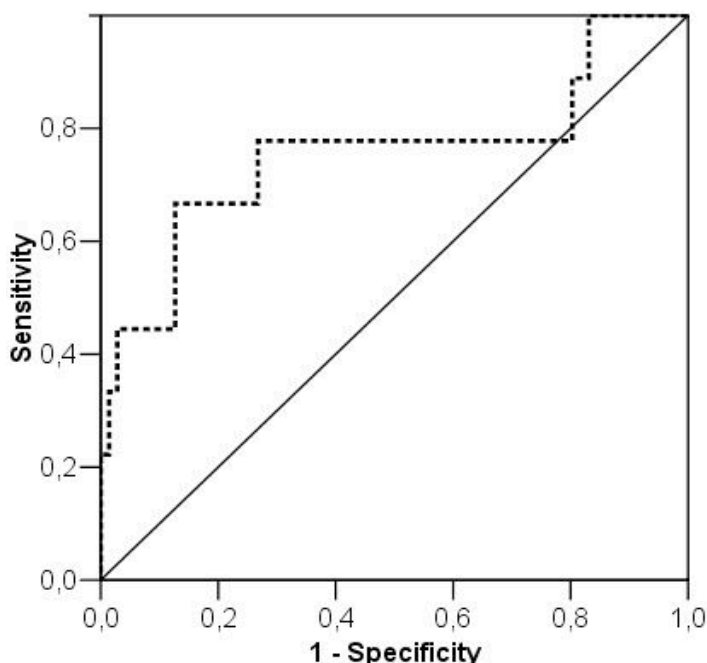
## RESULTS

Out of the 80 patients, 20 suffered from microbiological sepsis, 40 from clinical sepsis and 20 had SIRS due to non-infective causes. There were no differences among the three groups, regarding age, gender, SOFA score and Charlson index. Overall mortality rate was 11.25%, with 3 events each in microbiological sepsis, clinical sepsis and SIRS ( $P = \text{n.s.}$ ).

The most common sites of infection were the respiratory tract (67%), the urinary tract (17%), abdomen (5%), skin (8%) and others (3%). Characteristics of the population are reported in Table 1. Ang-2 level significantly correlates with 7 days-mortality, regardless of diagnosis of SIRS or sepsis (Fig. 1) (AUC 0.756, 95%CI 0.544-0.967) and is significantly correlated with hemostasis, kidney and lung dysfunction (Table 2). Patients with plasma values above 15 $\mu\text{g/mL}$  have an increased risk of death (OR 13.3, 95%CI 2.6-71), respiratory tract (OR 71, 95%CI 6-790), and kidney (OR 77, 95%CI 8-735) complication and all three hemostasis complications had Ang-2 levels above 15 $\mu\text{g/mL}$  (Table 2).

## DISCUSSION

A wide array of inflammatory stimuli activate angiotensin-Tie2 system, which, in turn, triggers hyperpermeability, apoptosis and increases the response to vasoreactive stimuli. Increased permeability is a key factor for organ failure and shock and, independent of the initiating cause, it might represent a prognostic factor for risk stratification of patients with sepsis/SIRS. So far, Ang-2 has been mainly studied as biomarker for early diagnosis of sepsis in pediatric or adult ICU while the role as prognostic marker in medical ward patients has never been investigated, in spite of a solid rationale. In the present study, higher Ang-2 plasma levels are associated to substantial increased risk of death, hemostasis (mainly disseminate, intravascular coagulation), kidney and respiratory tract dysfunction. The results corroborate recent experimental data and further support the idea that Ang-2 is a key factor of SIRS/sepsis severity. This study holds some



**Fig. (1).** ROC curve for death and Ang-2.

messages. The first is that a bedside evaluation of Ang-2 would be helpful in prognostic stratification, the second that antagonists of Ang-2 could be a target for innovative approach to these patients [13] and, third, that beyond survival and lung injury, also kidney complication or DIC occurrence can be predicted by Ang-2 levels. The present study investigates SIRS/sepsis medical ward patients, which are usually disregarded in previous studies but, indeed, account for patients who might benefit from a more intensive care.

Beyond the cross sectional design, preventing the investigation of Ang-2 kinetics, this study has other limitations: the small sample size and the lack of comparison with other settings (paediatric and adult ICUs), thus limiting the application of the present data. Finally, as for others endothelial biomarkers, and according to Xing *et al.* [8], Ang-2 requires a careful assay standardization, and validation in large prospective studies to confirm its role as early prognostic marker in medical patients with SIRS/sepsis.

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

### ACKNOWLEDGEMENTS

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