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Ang-2, Tie-2, and Ang-2/Tie-2 ratio serum levels as diagnostic and prognostic biomarkers of sepsis in critically ill patients

Evaluarea nivelelor serice ale Ang-2, Tie-2 și a raportului Ang-2/Fie-2 ca biomarkeri pentru diagnosticul și prognosticului sepsisului la pacientul critic

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Abstract

Sepsis represents one the main cause of death in patients admitted to the intensive care. Biomarkers offer an alternative approach to the diagnostic and prognostic evaluation and improve the outcomes. Angiopoietin 2 (Ang-2) and Tyrosine kinase 2 (Tie-2) are biomarkers which may be involved in sepsis, Ang-2 being responsible for vascular remodelling while Tie-2 is their endothelial receptor.

The aim of the study: *To assess the Ang-2, Tie-2 and Ang-2/Tie-2 ratio serum levels in septic and non-septic patients and to investigate the independent value of circulating Ang-2, Tie-2, and Ang-2/Tie-2 ratios as predictors of prognosis in critically ill medical patients.*

Study design: *The study included 74 adults admitted to an intensive care unit (ICU). The patients were separated in two groups: Group A [sepsis: n=40] and Group B [no-sepsis: n= 34] patients. Serum levels of Ang-2 and Tie-2 were determined in the first 12 hours after admission and were correlated with ICU severity scores, APACHE II, SOFA and SAPS and with the death rate.*

Results: *Group A gave significantly higher values (p=0.01), for serum Ang-2 (11.07±9.21 ng/ml) compared to Group B (6.18±5.28 ng/ml). The level of Tie-2 was also higher (11.03±5.12 ng/ml) in Group A compared to Group B (9.46±4.99 ng/ml) (p=0.19). In Group A, the Ang-2/Tie2 ratio showed higher values than Group B (p=0.02). There was a positive association between severity scores (APACHE II, SAPS, and SOFA) and Ang-2, and Ang-2/Tie-2 ratio, but not for Tie2.*

Conclusions: *In our study Ang-2 and Ang-2/Tie-2 ratio serum levels had independent diagnostic value in patients with sepsis, as measured on admission.*

Keywords: *Ang-2, Tie-2, biomarker, sepsis, critically ill.*

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Rezumat

Sepsisul reprezintă una dintre patologiile cele mai frecvent intâlnite la pacienții din terapie intensivă. Peste 60% dintre aceștia prezintă hemoculturi negative și lipsa evidenței clare a infecției. Biomarkerii reprezintă o alternativă pentru stabilirea diagnosticului și determinarea prognosticului sepsisului la pacienții internați în terapie intensivă. Angiopoietina 2 (Ang-2) și Tirozinkinaza 2 (Tie-2) sunt biomarkeri care par să aibă implicație în sepsis: Ang-2 este responsabilă pentru remodelarea vasculară, iar Tie-2 este receptorul de care aceasta se leagă.

Obiective: *Evaluarea nivelelor serice ale Ang-2, Tie-2 și a raportului Ang-2/Tie-2 la pacienții septici și non-septici, precum și stabilirea rolului acestora ca predictori ai evoluției pacienților critici din terapie intensivă.*

Material și metodă: Studiul a inclus un număr de 74 pacienți adulți internați în terapie intensivă. Pacienții au fost grupați în: grupul A (septic) și grupul B (non-septic). Nivelele serice ale Ang-2 și Tie-2 au fost determinate în primele 12 ore după internare și au fost corelate cu scorurile de severitate, APACHE II, SOFA și SAPS, precum și cu rata mortalității.

Rezultate: *Grupul A a prezentat valori semnificativ mai crescute (p=0.01) ale nivelelor serice ale Ang-2 (11,07± 9,21 ng/ml), în comparație cu grupul B (6,18±5,28 ng/ml). Nivelele serice ale Tie-2 au fost de asemenea mai crescute pentru grupul A (11,03±5,12 ng/ml) comparativ cu grupul B (9,46±4,99 ng/ml) (p=0,19). Raportul Ang-2/Tie-2 a prezentat valori mai crescute în grupul A, comparativ cu grupul B (p=0,02). S-a observat o corelație pozitivă între scorurile de severitate (APACHE II, SAPS and SOFA) și nivelele serice ale Ang-2 și Ang-2/Tie-2, dar nu și cu Tie-2.*

Concluzii: *În studiul nostru, nivelele serice ale Ang-2 și raportului Ang-2/Tie-2 măsurate la internare par să aibă valoare diagnostică pentru sepsis. Ang-2 și raportul Ang-2/Tie-2 au capacitatea de a stabili prognosticul pacieților comparativ cu scorurile APACHE II, SAPS și SOFA. Tie-2 nu a prezentat valoare diagnostică pentru sepsis, dar poate fi utilizat ca predictor al mortalității.*

Cuvinte cheie: *Ang-2, Tie-2, biomarker, sepsis, pacient critic.*

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Introduction

Sepsis is a pathology frequent encountered in intensive care units [ICU], defined as the combination of an infection with two or more criteria for the systemic inflammatory response syndrome (SIRS) [1] and together with severe sepsis and septic shock are among the most frequent reasons for admission to an intensive care unit (ICU) [2]. The Surviving Sepsis Campaign (SSC), was conducted with the aim of establishing new criteria for defining sepsis and septic shock, and their guidelines define sepsis as "the presence, probable or documented, of infection together with systemic manifestations of infection" [3, 4].

Evidence of organ dysfunction may also be present in patients with severe sepsis [2], with delays in treatment predisposing to increased mortality; on the other hand, early diagnosis and the administration of effective antibiotic therapy may improve the survival rate of septic patients [3].

A broad range of clinical and laboratory parameters define the diagnostic standard of severe sepsis and septic shock [2]. Blood culture remains the gold standard method for detecting the presence of microorganisms in the bloodstream but usually requires several days for results to be known, as well as being plagued by some false negative cases, especially in patients undergoing antibiotic therapy [5]. Moreover, sepsis may be suspected in the absence of an obvious infectious source, and differentiating sepsis from non-infectious triggers of SIRS is difficult [6].

Sensitive biomarkers are required for early diagnosis and as indexes of prognosis. Increas-

ingly, full blood count (FBC), or cerebrospinal fluid (CSF) analysis are conducted in combination with the procalcitonin test, the C-reactive protein (CRP) test or the novel CD14 subtype (sCD14-ST) test. Procalcitonin (PCT) has emerged as the promising sepsis biomarker because of its close correlation with infections, and is considered an advance on C-reactive protein and other traditional markers of sepsis [7], though the diagnostic value of serum PCT concentrations for discriminating among SIRS, sepsis, severe sepsis, and septic shock remains to be established [8]. C-reactive protein (CRP) is one of the acute phase proteins synthesized by the liver. It has an excellent sensitivity but a very poor specificity for bacterial infections though the combinations of CRP with Lym C and PLTC was considered as useful in determining the likelihood of sepsis [9].

Soluble CD14 subtype (sCD14-ST), also known as presepsin, is a novel and promising biomarker with a higher sensitivity and specificity in the diagnosis of sepsis, [10] and in patients with suspected severe sepsis and septic shock, presepsin is considered to be a valuable diagnostic tool for assessing the severity of sepsis compared to PCT, IL-6, CRP and WBC [11].

In sepsis, angiopoietins have been associated with vascular leakage, inflammation, and break- down to the microvascular endothelium. Angiopoietins (Ang-1 and Ang-2) are antagonistic factors in endothelial cell activation [12], and though reported studies refer mostly to proliferative diseases [13, 14] they are also associated with inflammation [15, 16].

Increased plasma Ang-2 levels are reported in critically ill patients with sepsis and are associated with increased hospital mortality [17, 18, 19, 20, 21], as well as being correlated with the severity of illness [16, 22].

Current research suggests that Ang-2 is involved in the onset of septic shock and that fever and increased Ang-2 levels correlate with an increased prospect of developing septic shock, and generally, studies conclude that Ang-1 and Ang-2 levels may be a useful biomarkers in patient prognosis and a valuable tool in early decision therapies [12]. Currently available evidence suggests that the Ang-2/Tie-2 ratio is changed in patients with sepsis [17], though more associated data on Ang- 2/Tie-2 responses are required [16].

According to the review of the literature, Ang2 in combination with Tie 2 have been little discussed as biomarkers of sepsis. Therefore due to the complex pathophysiology of sepsis, success will be better attained by not just looking at one particular biomarker but more likely a combination of several different biomarkers and the present study is intended to ascertain the role of Ang-2, Tie-2 and Ang-2/Tie-2 ratio serum levels in septic and non- septic critically ill medical patients, with the objective to use them in combination with other accepted biomarkers.

Therefore, the aims of present study are:

1. To assess the Ang-2, Tie-2 and Ang-2/Tie-2 ratio serum levels in septic and non- septic critically ill medical patients.

2. To investigate the independent value of circulating Ang-2, Tie-2, and Ang-2/Tie- 2 ratios as predictors of prognosis in critically ill medical patients.

Null hypotheses:

1. There is no difference in the Ang-2, Tie-2 serum levels, and Ang-2/Tie-2 ratio, in septic and non-septic critically ill medical patients.

2. There is no correlation between the independent value of circulating Ang-2, Tie- 2 serum levels and Ang-2/Tie-2 ratio as predictors of severity of illness or outcome in critically ill medical patients.

Methods & Materials

All adult patients admitted to the Intensive Care Unit (ICU) of the Clinical County Hospital in Tîrgu Mureş, from January 1st to 31st July 2014, underwent routine clinical examination, including vital function monitoring (ECG, BP, and SpO2) and standard laboratory test. This resulted in 311 patients being included in the study. From this cohort, patients who were most likely to present with elevated levels of Ang-2 or Tie-2 due to co-morbidities such as trauma, cardiac arrest in the last 72 hours, hematologic disorders, tumours or uncontrolled diabetes, were excluded. All patients were assessed for the presence of SIRS criteria and infections. Where the presence or absence of sepsis could not be clearly ruled out, these patients were also excluded. This resulted in two groups. Group A (Sepsis: n=40) and Group B (No-sepsis: n=34). Group B acted as a control.

A diagnosis of "sepsis" was made if there was an identification of SIRS in the presence of infection. The identification of SIRS was accepted if at least two of the following criteria were met.

- Temperature >38.3°C] or <36°C.
- Heart rate over 90 bpm.
- Respiratory rate >24 breaths/min or mechanically ventilated.
- Systolic blood pressure under 90 mmHg after fluid resuscitation.
- Total white cell count > 12,000 /μL or < 4000 /μL.

The proof of infection was established by positive blood cultures or the clinical evidence of a source of infection such as pus in bronchial secretions or pus associated with wounds or other organs.

The approval of the local Ethics Committee was previously obtained, and all patients or their relatives gave their written informed consent before study being conducted.

Within the first six hours after admission, a medical history was taken by the admitting physician including descriptive data consisting of demographics, diagnosis, clinical data, and disease severity scores. The recorded data included blood pressure, SpO2, ECG, ventilation parameters such as current volume, respiratory rate and ventilation mode and diuresis. Additionally, the following laboratory tests were performed: blood cell count [leucocytes, platelets, and erythrocytes], creatinine, urea, bilirubin, International Normalized Ratio [INR] and liver enzymes [AST, ALT, and GGT].

APACHE II, SOFA, and SAPS II severity scores were also calculated.

Following group allocation, blood samples for quantitative Ang-2 and Tie-2 were collected directly in serum tubes without anticoagulant via vascular puncture. The samples were centrifuged and the serum collected and frozen at -70°C for later processing. Blood cultures were determined using separate vials for aerobic [Standard SA] and anaerobic [Standard SN] organisms. Analysis of the blood cultures were processed using the BacT/Alert 3D [Biomerieux, France] automated haemoculture system and serum expression of Ang-2 and Tie-2 was evaluated using the Enzyme Linked Immuno-Sorbent Assay [ELISA] test [R&D Systems, Minneapolis, USA]. Sensitivity for ELISA analysis kit was 0.066 ng/ml for Tie-2, and 21.3 pg/ml for Ang-2. Variation coefficients for ELISA kits were: Ang-2 – intra-assay 6.5%, Tie-2 – intra-assay 4.3%.

Statistical analysis was performed using Microsoft Excel [Microsoft, Washington, USA], GraphPad [GraphPad Software, Inc., California, USA] and MedCalc [MedCalc Software, Ostend, Belgium].

Logistic binomial regression calculations were developed to analyse the relationship be-

tween the presence of sepsis and mortality for the independent variables, Ang-2, and Tie-2. The odds ratio for Ang-2 and Tie-2 for predicting sepsis and death were calculated.

The analysis included sensitivity and specificity of Ang-2 and Tie-2 for the diagnosis of sepsis and mortality. Their correlation with APACHE II, SOFA, SAPS II scores, the presence of sepsis, PCT, CRP, positive haemocultures was analysed.

The accuracy of the Ang-2 and Tie-2 to discriminate septic cases from normal cases was evaluated using receiver-operating-characteristic [ROC] curve analysis, with determination of area under the curve [AUC], the significance level were calculated for the sensitivity and specificity, and the cut-off value were established for both Ang-2 and Tie-2 for sepsis diagnosis.

The Kolmogorov-Smirnoff test was used to determine whether data followed normal distribution and Bartlett's Test to assess the assumption of equal variances. Where applicable the Mann-Whitney or Pearson tests were used for variable correlations. Pearsons`s chi^2 test with Fisher or Yates correction were used for comparing the distribution of nominal values. The level of significance was set at $\alpha = 0.05$.

A sample size and power calculation was undertaken using NCSS.PASS 13 Statistical Software (Kayswille, Utah, USA).

Results

Forty patients [54%] met the stated criteria for sepsis. Of those, ten [25%] presented with at least one positive haemoculture. Five patients from Group A [sepsis] and one patient from Group B [no-sepsis] had a positive haemoculture of *Staphylococcus Epidermidis*.

No significant differences were reported between Group A [sepsis] and Group B [no- sepsis] regarding their demographic characteristics, except for gender, were Group A showed a preponderance of males [**Table 1**].

Table 1. Demographic and clinical characteristics of Group A [Sepsis] & Group B [No-Sepsis]

Parameter	Group A [Sepsis] **[n=40, 54%]**	Group B [No-Sepsis] **[n=34, 46%]**	**P value**
Age [years]^A	76 [15.87]	68 [13.39]	0.05*
Gender, male [n, %]	24 [60%]	12 [35.29%]	0.04
BMI [kg/m^2]^A	24.6 [8.34]	26.9 [8.77]	0.13*
Days in intensive care unit	2 [4.5]	10 [10.5]	< 0.01
Days under vasoactive treatment	1 [1.75]	3 [3.5]	0.01
Mortality [n, %]	32 [80%]	24 [70.59%]	0.42*
Survival time [days]^B	3 [2-6]	12 [6-30]	0.02
APACHE II [val]^A	27 [8.8]	23 [7.7]	0.02
SOFA [val]^A	9 [3.6]	7 [3.1]	0.06*
SAPS [val]^A	51.5 [20.2]	42 [14.8]	0.03

Superscript^A = [mean ± SD]
Superscript^B = [median; 95% confidence intervals]
Mann-Whitney test [significant: p < 0, 05].
*Superscript * NOT significantly different.*

Group A spent significantly fewer days in the ICU and shorter survival time. [p=0.02]. Three patients in Group A [7.5%] had a gram positive infection and ten patients [25%] had gram-negative infections. Three patients were identified with more than one organism [**Table 2**].

The combinations were:

- *Staphylococcus aureus* MRSA [Methicillin resistant] with *Pseudomonas aeruginosa,*
- *Staphylococcus aureus* MRSA with *Klebsiella pneumoniae*,
- *Pseudomonas aeruginosa* with *Klebsiella pneumoniae*; and *Acinetobacter baumannii*. [**Table 2**].

The serum levels of Ang-2 and Tie-2 showed a significantly higher level of Ang-2 for Group A in comparison with the control Group B [p=0.01].

The serum levels of Tie-2 was also higher in Group A compared to the control Group B, but this was not significant [p=0.19]. The Ang-2/Tie-2 ratio for Group A in comparison with the control Group B was also significant higher [p=0.02]. [**Figure 1, Table 3**].

The logistic regression analysis for the presence of sepsis showed a prediction value for Ang-2 (p=0.02) with an odds ratio of 1.12; while for Tie-2 was not significant (p=0.19) with an odds ratio of 1.07.

The logistic regression calculation for the outcome (death) indicated no significant predic-

Table 2. Positive haemocultures from Group A [Sepsis]

Organisms	No	%
Gram positive	**3**	**7.5**
Methicillin-resistant Staphylococcus aureus [MRSA]	2	5
Enterococcus faecium	1	2.5
Gram negative	**10**	**25**
Acinetobacter baumannii	2	5
Klebsiella Pneumoniae ESBL	3	7.5
Pseudomonas aeruginosa	2	5
Escherichia coli ESBL	2	5
Stenotrophomonasmaltophilia	1	2.5
Contamination	**5**	**12.5**
Staphylococcus epidermidis	5	12.5
Negative	**30**	**75**
Total	**40**	

ESBL - extended-spectrum beta-lactamase; MRSA - Methicilin Resistant Staphylococcus Aureus.

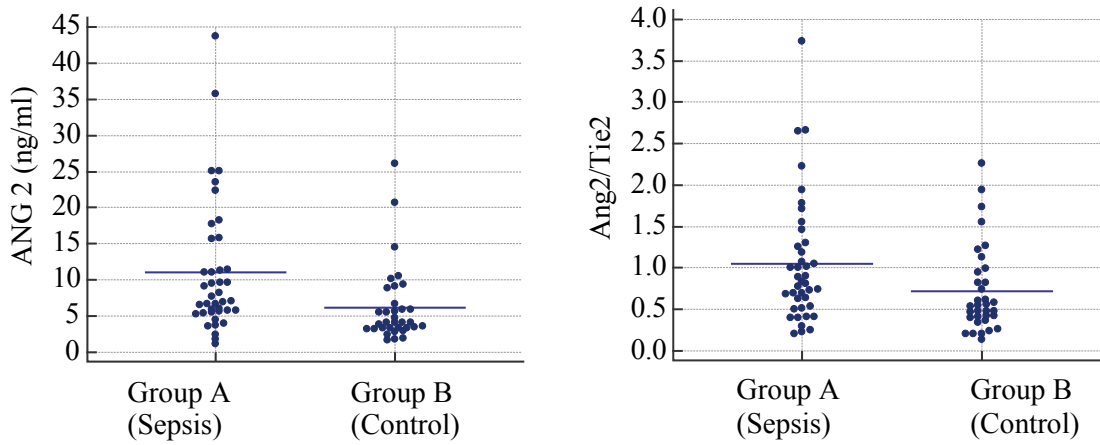


Figure 1. The Ang-2 and Ang-2/Tie-2 ratio serum levels in Group A [Sepsis] & Group B [no Sepsis]

tion value for both Ang-2 (p=0.1523) and Tie-2 (p=0.57) with and Odds ratio of 1.08 for Ang-2 and 0.97 for Tie-2.

The sensitivity of Tie-2 for predicting the outcome of the patients was very high [91% for mortality], but with low specificity [28%].

The multivariate correlation analysis between biomarkers and severity score revealed a significant positive association between all severity scores [APACHE II, SAPS and SOFA] and Ang-2 [p=0.04, <0.01, 0.03], and Ang-2/Tie-2 ratio [p=0.01, <0.01, 0.01].

Tie- 2 alone PCT and CRP were not significantly correlated with APACHE II, SAPS, and SOFA [**Table 4**].

Table 3. Seric levels of Ang-2 and Tie-2 in Group A [Sepsis] & Group B [no Sepsis]

	Ang-2 Group A	Ang-2 Group B	Tie-2 Group A	Tie-2 Group B
Number of values	40	34	40	34
Minimum	1.101	1.667	4.08	3.92
Median	7.367	4.106	9.81	8.54
Maximum	43.76	26.14	25.57	30.49
Mean	11.07	6.182	11.03	9.461
Std. Deviation	9.216	5.287	5.129	4.996
Std. Error of Mean	1.457	0.9067	0.8109	0.8567
Lower 95% CI of mean	8.121	4.337	9.391	7.718
Upper 95% CI of mean	14.02	8.026	12.67	11.2

Table 4. Correlation between biomarkers and clinical severity scores in Group A [Sepsis] & Group B [No-Sepsis]

		Apache_II	SAPS	SOFA	Sepsis	Bloodcult*	Mortality
Ang-2	CC	0.23	0.33	0.26	0.31	0.06	0.16
	p	0.04	< 0.01	0.03	0.01	0.60	0.17
Tie-2	CC	-0.07	-0.07	-0.07	0.16	0.03	< 0.01
	p	0.54	0.58	0.54	0.19	0.79	0.99
Ang-2/Tie-2	CC	0.29	0.34	0.30	0.24	0.01	0.13
	p	0.01	< 0.01	0.01	0.04	0.93	0.27
CRP	CC	0.09	0.10	-0.03	0.41	-0.05	-0.05
	p	0.44	0.40	0.82	< 0.01	0.68	0.68
PCT	CC	0.15	0.18	0.04	0.44	-0.07	0.13
	p	0.22	0.13	0.74	< 0.01	0.56	0.28

CC - Pearson correlation coefficient;
p - p value;
** - Positive blood cultures.*

In Group A, both PCT and CRP showed significant raise in serum levels [**Figure2**].

The overall mortality for the patients in the first 60 days was 75.7%. The survival rates at 60 days were 20% [n=8] in Group A and 29.4% [10] in Group B. There was a significant difference between the two groups with higher mortality rate and shorter survival times for Group A [p=0.0169]. All patients from Group A died within the first ten days following admission to the ICU.

Multivariate analysis did not reveal any significant correlation between the levels of the biomarkers for sepsis and mortality [Ang-2 p=0.17, Tie-2 p=0.99, PCT p=0.28, CRP p=0.68].

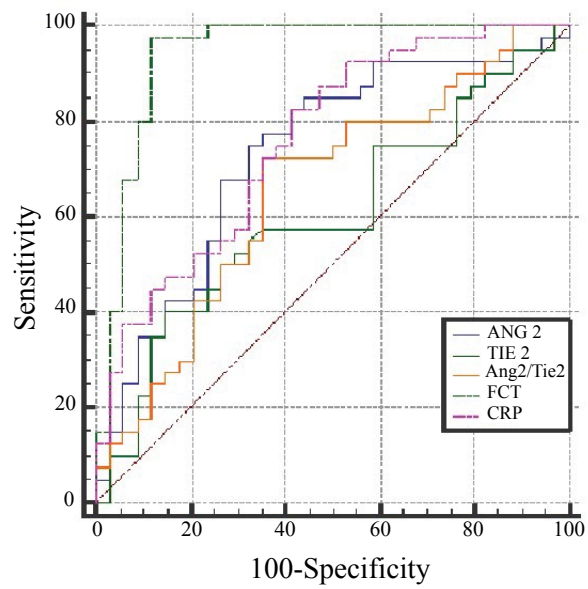
The results of the sample size and power calculation indicated that for Ang-2 the power of statistical data was 0.99 and for Tie-2 was 0.68.

The biomarkers did not prove to be significantly different for bacteraemia patients.

Discussion

Sepsis represents one the main cause of death in patients admitted to the intensive care [3]. However, a review of the literature showed that about 40 to 60% of the septic patients had negative blood cultures and lack of microbiological evidence of the infection [23, 24, 25,26]. Biomarkers offer an alternative approach to the diagnostic and prognostic evaluation of the septic patient, and there is an increasing interest to use them in clinical practice [27, 28, 29].

Though there were more males in Group A, no significant differences were found between



	Sensitivity	Specificity	AUC [SE]	Cut off value	p
Ang-2	75.00	67.65	0.731 [0.06]	5.61	0.01
Tie-2	40.00	85.29	0.603 [0.07]	13.32	0.12
Ang-2/Tie-2	72.50	64.71	0.656 [0.06]	0.62	0.02
PCT	97.50	88.24	0.939 [0.03]	0.20	0.01

Figure 2. ROC curves for Ang-2, Tie-2, Ang-2/Tie-2, PCT and CRP for sepsis

females and males between Group A and Group B, and it was concluded that this difference did not affect the outcome of the study.

The days admitted in intensive care and the days under vasoactive treatment are significant shorter for Group A because the survival time was also much shorter. 80% of Group A (n=32) died in the first ten days compared to a survival rate of 58.8% (n=20) in Group B.

Regarding the positive blood cultures, 50% (n=5) of patients infected with MRSA, *Acinetobacter baumannii*, *Klebsiella pneumoniae* ESBL and *Pseudomonas aeruginosa*, it was considered that these were due to nosocomial hospital infections. None of those patients survived, the high mortality rate (100%) reveals the severity of this type of infection. All five patients were ventilated mechanically, the lungs being the site of infection. Ventilator acquired pneumonias are a common cause worldwide of ICU infections [30, 31, 32]. The positive cultures with *Staphylococcus Epidermidis* were deemed to be due to contamination during the blood sample handling and excluded from positive blood culture patients.

The serum values of Ang-2 in Group A was significantly higher (11.07 ng/ml) compared to Group B (6.18 ng/ml).

The serum values of Tie-2 values were also higher in Group A (11.03 ng/ml) compared to Group B (9.46 ng/m), but were not significant (p=0.13).

The Ang-2/Tie-2 ratio was also significantly higher in Group A (p=0.02), and this is in agreement with former studies [20, 33].

The aims of the study were to assess Ang-2, Tie-2, and Ang-2/Tie-2 ratio serum levels in septic and non-septic in critically ill medical patients and to investigate the independent values of circulating Ang-2, Tie-2, and Ang-2/Tie-2 ratios as predictors of prognosis in critically ill medical patients. The null hypotheses were that there is no difference in the Ang-2, Tie-2 and Ang-2/Tie-2 ratio serum levels in critically ill patients with sepsis and those how do not present with no-sepsis. There is no correlation between the independent value of circulating Ang-2, Tie-2, and Ang-2/Tie-2 ratio serum as predictors of severity of illness or outcome in critically ill medical patients. As the serum levels of Ang-2 showed a significantly higher level of Ang-2 for Group A in comparison with the control Group B [p=0.01], and the Ang-2/Tie-2 ratio for Group A in comparison with the control Group B was also significant higher [p=0.02]. However, the serum values of Tie-2 did not prove to be different between the two groups [p=0.13]. Therefore, the first of the null hypotheses is party refuted.

The biomarkers' ROC indicated that Ang-2 [AUC=0.731] and Tie-2 [AUC=0.603] had a lower sensitivity [power] to determine sepsis in comparison with PCT [AUC=0.939] [**Figure 1**].

The correlation between Ang-2 and sepsis is in accordance with previous reports [22]. Data from a previous study [34] report a decrease in Tie-2 renal tissue levels in sepsis patients who succumbed to the condition.

Multivariate analysis did not revealed any significant correlation between the levels of the biomarkers Ang-2, Tie-2 and Ang-2/Tie-2 for outcome [mortality] [Ang-2 p=0.17, Tie-2 p=0.99, PCT p=0.28, CRP p=0.68]. The Ang-2 serum levels and Ang-2/Tie-2 ratio in the Group A and Group B presented a positive correlation with the APACHEII, SAPS, SOFA illness severity scores, though this was not true of the Tie-2 serum levels. The second of the hypotheses is therefore partly upheld.

The overall mortality and the length of ICU admission of group A were higher, because, due to the severity of their condition, they died earlier than the control group.

APACHE II was the severity score used in the clinic from which our sample was obtained,

and, therefore, other scores could not easily be incorporated at the time of the study.

In agreement with the present results, several studies exhibited a positive correlation between Ang-2 serum levels and the severity of the sepsis [18, 21]. A statistically significant association between serum levels of Ang-2 and mortality was not demonstrated in the present study, despite this being reported in other studies [29]. This may partly be due to the high mortality in both groups of patients, in the current study.

No relevant studies were identified in the literature with which the data on Tie-2 serum levels could be validly compared.

The results of the study denote the possibility of clinically determining the "sepsis patient" based on Ang-2 serum values. Furthermore, the positive correlation between Ang-2 and severity scores endorses the use of Ang-2 serum values for predicting the severity of the sepsis.

For diagnostic and prognostic purpose, PCT is more useful than C-reactive protein and other markers of sepsis but is not accurate enough for clinicians to establish the precise diagnosis of sepsis. The advantage of PCT is augmented by the early increase in serum levels after infectious challenge, within the first three to six hours [29, 30, 35].

Early PCT levels in sepsis are not accepted as reliable as a diagnostic biomarker. The combination of several biomarkers may reduce the limitations of sensitivity and specificity inherent in the use of single biomarkers and this is the main reason the current study investigated the roles of Ang-2 and Tie-2, without the aim of making direct comparisons of different markers [8,12, 36].

PCT values below 0.25 µg/L in patients with signs of infection indicate a low probability of positive blood culture, whereas PCT values above 0.25 µg/L are frequently correlated with positive blood culture results. In sepsis PCT levels are mostly greater than 1-2 µg/L and often reach values over ten µg/L, or higher in individual cases, enabling the diagnostic differentiation between different clinical conditions from bacterial infection [29, 30,35].

In case of viral infections, chronic inflammatory processes or autoimmune diseases PCT levels are usually low.[37] Studies suggest that PCT may have role in reducing antibiotic exposure for critically ill patients. For intensive cares intending to integrate PCT analysis for routine clinical practice, the cost-effectiveness is likely is correlated with pre-implementation length of an antibiotic and the presence of the antibiotic resistance for the ICU [37].

Sepsis is an inflammatory process caused by an infection and, therefore, there is no very clear demarcation between the "sepsis" and "non-sepsis" patient seen in an ICU. All patients admitted to an ICU are likely to present with some degree of microbiological presence, even if they are not infectious, due to having catheters and invasive monitoring methods as part of their ongoing treatment. The fundamental objective is to distinguish between patients whose treatment regime targets the management of organ function compared to those who require antibiotic therapy to combat an infection.

Ang-2 serum values were significantly higher in the Group A. The ROC curves [Figure 1] indicate that the calculated cut-off of 5.61 ng/ml value best separates the "sepsis" from the "non-sepsis" patient. Tie-2: There was no statistically significant difference in Tie-2 serum levels in the two groups, Tie-2 are the receptors to whom the Ang-2 is bounded, which results in no down-regulation or up-regulation in Tie-2 receptor serum values for sepsis patient. It was concluded that Tie-2 is not a suitable or useful biomarker for sepsis. However, the range from 3.92 to 30.99 ng/ml suggests that a refining of the cases is indicated in order to establish the pa-

rameters or conditions existing when there is a regulation of these receptors. Another study may be useful for this.

Ang-2/Tie-2 ratio: There was a significant difference in the Ang-2/Tie-2 ratio between Group A and Group B, the ratio being higher in Group A with a cut-off value of 0.62. Never the less the Ang-2 serum levels suggested a higher diagnostic value for the Ang-2/Tie-2 ratio and therefore, according to the results of the present study, are the preferred sepsis biomarker. An explanation of the Ang-2/Tie-2 ratio being significantly different in the two groups depends as much on the Ang-2 serum level being higher for sepsis and the Tie-2 serum levels being the same in both groups than Tie-2 being a primary biomarker.

As a possible algorithm, it is suggested to use the Ang-2 assessment in combination with PCT for patients in whom the SIRS criteria is present, but an infectious process isn't proved. Thus, the Ang-2 enhances the probability of the presence of sepsis together with PCT and allows stratifying the severity of patients condition, which cannot be performed using PCT alone.

To be clinically useful, prospective biomarkers should be practical, both economically and otherwise in the clinical setting. They should be easily adapted to practice and effortlessly and routinely used in patient diagnosis and treatment.

Conclusions

The results showed that Ang-2 and Ang-2/Tie-2 ratio serum levels had independent diagnostic value in patients with sepsis, as measured on admission.

Additionally, Ang-2 and Ang-2/Tie-2 ratio serum levels had a valuable capacity to predict patient evolution when compared with APACHE II, SAPS, and SOFA scores. Moreover, Tie-2 alone proved to have no significant values for the diagnosis of sepsis, but can predict the mortality for critically ill patients.

The studied biomarkers did not show very high sensitivity nor specificity for sepsis. Therefore, it is not recommend that this be used alone though it does have the potential to be used in combination of other biomarkers.

Conflict of interest

The authors have no conflict of interest regarding developing this study.

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Abbreviations

APACHE II - Acute physiology and chronic health evaluation
AUC - Area under curve
BMI - Body mass index
CC - Correlation coefficient
CI - Confidence Interval
CRP - C-reactive protein
DIC - Disseminated intravascular coaguolpathy
ELISA - Enzyme-linked immunosorbent assay
ESBL - Extended spectrum beta-lactamase
MRSA - Methicilin-resistant Staphylococcus aureus
PCT - Procalcitonin
ROC - Receiver operating characteristic
SAPS - Simplified acute physiology score

SE - Standard error
SD - Standard Deviation
SIRS - Systemic inflammatory response syndrome
SOFA - Sequential Organ Failure Assessment
TIE - Tyrosine kinase
VEGF - Vascular endothelial growth factor

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