The Role of Angiopoietine-2 in the Diagnosis and Prognosis of Sepsis

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ABSTRACT

Introduction: Angiopoietin-2 (ANG-2) is a new biomarker whose blood-serum values increase in sepsis and its expression is elevated in line with the severity of the degree of inflammation. The aim of this study was to identify the diagnostic role of ANG-2 in patients with non-surgical sepsis admitted to an intensive care unit.

Material and methods: This was a prospective randomized study including 74 patients admitted in the Clinic of Intensive Care of the County Clinical Emergency Hospital Tirgu Mures, divided into two groups: Group S: patients with sepsis (n=40, 54%) and Group C: control, without sepsis (n=34, 46%). ANG-2 levels were determined in both groups.

Results: From the Group S, 14 patients (35%) had positive haemocultures. ANG-2 values varied between 1 and 43 ng/mL, with an average of 6.0 ng/mL in patients without sepsis and 10.38 ng/mL in patients with sepsis (p=0.021). A positive correlation between ANG-2 and SAPS II, SOFA and APACHE II severity scores was demonstrated, as was a positive correlation between serum levels of ANG-2 and procalcitonine. ANG-2 had a 5.71% specificity and 74.36% sensitivity for diagnosis of sepsis.

Conclusions: ANG-2 serum levels were elevated in sepsis, being well correlated with PCT values and prognostic scores. ANG-2 should be considered as a useful biomarker for the diagnosis and the prognosis of this pathology.

Keywords: Angiopoietin-2, sepsis, infection

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INTRODUCTION

Sepsis is a pathology frequent encountered in intensive care units, occurring as a major complication in the development of serious infections. Severe sepsis, defined as a sepsis associated with organ dysfunction [1] is associated with a high mortality rate [2] and is caused by an infection induced immune response [3]. Diagnosis is based on clinical criteria, the principle indicator being a systemic inflammatory response syndrome (SIRS), triggered by an infection [4,5]. The prognosis for these patients is dependant on the early establishment of the proper diagnosis and the quick initiation of antibiotic therapy [6]. Recently, special attention has been given to new biomarkers associated with sepsis. Some of these have proved to be effective in the diagnosis and prognosis of this severe disease. Angiopoietin-2 (ANG-2) is one of these biomarkers, and has been intensively studied. It is produced by endothelial cells from Weibel-Palade corpuscles. ANG-2 induces inflammation of endothelial cells, as well as vessel regression and destruction. The blood-serum values of ANG-2 increase in sepsis and its expression is elevated in line with the severity of the degree of inflammation. However high expression of this biomarker is also found in other pathologies such as multiple myeloma [7], squamos cells carcinoma [8] or heart failure in patients on dialysis [9]. The mechanism of action of Angiopoietin-2 involves the tyrosine kinase receptor (TIE2) [10].

The aim of this study is to identify the diagnostic role of ANG-2 in patients with non-surgical sepsis ad-
mitted to an intensive care unit. We tested the hypoth-
thesis that ANG-2 has a high sensitivity and specificity
for the diagnosis of sepsis and for the prognosis of this
disease.

Objectives of the study were:
• To assess the prognostic role of ANG-2 in sepsis.
• To study the correlation between ANG-2 and pro-
calcitonine (PCT).
• To study the correlation between ANG-2 and the
following score systems:
  a. APACHE II score (Acute Physiology and
     Chronic Health Evaluation),
  b. SOFA (Sequential Organ Failure Assessment)
  c. SAPS II (Simplified Acute Physiology Score).
• To study the correlation between ANG-2 and re-
  quirements for vasoactive medication.
• To study the correlation between ANG-2 and re-
  nal function.

Material and Methods
This is a prospective randomized study including 74
patients admitted in the Clinic of Intensive Care of the
County Clinical Emergency Hospital Tirgu Mureș in
the period of January to November 2014. The study
was approved by the Ethics Committee of the Univer-
sity of Medicine and Pharmacy of Tirgu Mures and all
the patients or their relatives were given a clear expla-
nation of the study and gave their informed consent
before randomization.

Inclusion criteria: All patient, of either sex, admitted
to the intensive care ward, who were over the age of 18
years and had no associated pathology or had received
any surgical intervention in the previous 72 hours,
were admitted into the study.

Exclusion criteria: patients with cardiac arrest or
associated pathologies that could cause expression of
high levels of ANG-2.

On admission to the intensive care unit a medical
history was taken and the following tests carried out:
  a. record of status of vital signs
  b. laboratory tests including cell blood count, cre-
atinine, urea, coagulation parameters and liver
  transaminase

A clinical diagnosis was made at this point. Blood
cultures and bacteriological determinations were used
to confirm the diagnosis.

Blood samples were collected directly via vascular
puncture after skin disinfection with betadine. The
blood cultures were accomplished using separate vials
for aerob germs (Standard SA) and anaerob (Standard
SN). Analyses of the blood cultures were processed us-
ing the BacT/Alert 3D (Biomerieux, France) automat-
ed haemoculture system.

APACHE II, SOFA and SAPS II severity scores were
calculated and serum levels of PCT, C-Reactive pro-
tein (CRP) and ANG-2 were determined in the first 12
hours after admission.

The blood samples were frozen at -70°C and pro-
cessed later.

ANG-2 expression was evaluated using the Enzyme-
Linked Immuno Sorbent Assay (ELISA) test (R&D
Systems, Minneapolis, USA).

PCT, CRP, the sepsis-related organ failure assess-
ment was determined using the immunoturbidimetry
method (Cobas 6000, Roche Diagnostics, Germany)
and PromoKinekits for the detection, elimination and
prevention of cell culture contamination (PromoCell
GmbH, Heidelberg, Germany).

Patients were divided into two groups: Group S: pa-
tients with sepsis (n=40, 54%) and Group C: control,
without sepsis (n=34, 46%).

A diagnosis of sepsis to be accepted, all three of the
following criterion had to be present:
  a. Two clinical criteria for SIRS
  b. PCT higher than 0.2 ng/mL
  c. confirmed infection.

Statistical analysis was performed using Micro-
sot Excel (Microsoft, Washington, USA), GraphPad
(GraphPad Software, Inc., California, USA) and Med-
Calc (MedCalc Software, Ostend, Belgium).

Analysis included specificity and sensibility of
ANG-2 for diagnosis of sepsis, and correlation with the
above mentioned parameters and death. Graphic rep-
resentation of receiver-operating-characteristic (ROC)
were used, with determination of area under the curve
(AUC). Quantitative variables were tested for normal
distribution using the Kolmogorov-Smirnov test and
Bartlets Test for equal variances. Where applicable the
Mann-Whitney or Pearson tests were used for variable
correlations. Pearson’s chi² test with Fisher or Yates
correction were used for comparing distribution of
nominal values. A p value<0.05 was considered statis-
tically significant.
RESULTS

Seventy four patients were enrolled in the study, of which 40 patients (54%) fulfilled the criteria for sepsis, and 34 (46%) were included in the control group. From the Group S, 14 patients (35%) had positive haemocultures. ANG-2 values varied between 1 and 43 ng/mL, with an average of 6.0 ng/mL in patients without sepsis and 10.38 ng/mL in patients with sepsis (p=0.021) (Figure 1).

Demographic and paraclinical data of the patients in the two groups are presented in Table 1.

A positive correlation between ANG-2 and SAPS II, SOFA and APACHE II severity scores was demonstrated, as was a positive correlation between serum levels of ANG-2 and PCT (Table 2).

The lungs were the most frequent locus of infection in the study groups (Table 3).

ANG-2 was both “specific” and “sensitive” in the diagnosis of sepsis: 65.71% specificity and 74.36% sensitivity (Figure 2).

![Figure 1. ANG-2 values in the studied patients](Unauthenticated)

The cut-off value ANG-2 for diagnosis of sepsis was established at 5.61 ng/mL according to the Youden test.

A high mortality rate of 75.67% (n=56) was recorded in those cases where ANG-2 showed a high specificity (88.89%) together with a lower sensitivity (35.71%) (p=0.195) (Figure 3).

Table 1. Demographic and paraclinical data in the two groups of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group –S (Patients with sepsis) (n=40, 54%)</th>
<th>Group- C (Control. Patients without sepsis) (n=34, 46%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, average ± SD)</td>
<td>76 ± 15.87</td>
<td>68 ± 13.39</td>
<td>0.0525</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>24 (60%)</td>
<td>12 (35.29%)</td>
<td>0.0393</td>
</tr>
<tr>
<td>BMI (kg/m², average ± SD)</td>
<td>24.6 ± 8.34</td>
<td>26.9 ± 8.77</td>
<td>0.1300</td>
</tr>
<tr>
<td>Days in intensive care unit TI (average ± SD)</td>
<td>2 ± 4.6</td>
<td>10 ± 10.33</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Days under vasoactive treatment (average ± SD)</td>
<td>1 ± 1.73</td>
<td>3 ± 3.35</td>
<td>0.0073</td>
</tr>
<tr>
<td>Patients requiring vasoactive treatment (n, %)</td>
<td>33 (82.5%)</td>
<td>28 (82.23%)</td>
<td>0.434</td>
</tr>
<tr>
<td>Mortality (n, %)</td>
<td>32 (80%)</td>
<td>24 (70.59%)</td>
<td>0.4197</td>
</tr>
<tr>
<td>ANG-2 (ng/mL, average ± SD)</td>
<td>7.37 ± 9.21</td>
<td>4.11 ± 4.34</td>
<td>0.0005</td>
</tr>
<tr>
<td>PCT (ng/mL, average ± SD)</td>
<td>1.525 ± 3.03</td>
<td>0.71 ± 0.75</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CRP (ng/mL, average ± SD)</td>
<td>164.4 ± 126.7</td>
<td>82.71 ± 78.98</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Haemoglobin (g/dL, average± SD)</td>
<td>12.35 ± 3.09</td>
<td>11.75 ± 1.84</td>
<td>0.2762</td>
</tr>
<tr>
<td>Haematocrit (%, average± SD)</td>
<td>33.2 ± 8.99</td>
<td>34.05 ± 9.84</td>
<td>0.6879</td>
</tr>
<tr>
<td>Leucocytes (x1000/mm³, average± SD)</td>
<td>16.35 ± 12.44</td>
<td>13.95 ± 9.7</td>
<td>0.6027</td>
</tr>
<tr>
<td>Creatinine (mg/dL, average± SD)</td>
<td>1.84 ± 3.37</td>
<td>0.82 ± 1.88</td>
<td>0.0005</td>
</tr>
<tr>
<td>Urea (mg/dL, average± SD)</td>
<td>104.9 ± 98.21</td>
<td>62.95 ± 58.68</td>
<td>0.0168</td>
</tr>
<tr>
<td>APACHE II (val, average± SD)</td>
<td>27 ± 8.8</td>
<td>23 ± 7.74</td>
<td>0.0152</td>
</tr>
<tr>
<td>SOFA (val, average± SD)</td>
<td>9 ± 3.65</td>
<td>7 ± 3.07</td>
<td>0.0616</td>
</tr>
<tr>
<td>SAPS (val, average± SD)</td>
<td>51.5 ± 20.18</td>
<td>42 ± 14.77</td>
<td>0.0276</td>
</tr>
</tbody>
</table>

* p value calculated using Mann-Whitney test (significant for p<0.05).
Table 2. Correlations between ANG-2 and PCT levels, severity scores, days of vasoactive medication and renal function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient *</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT</td>
<td>-0.3049</td>
<td>0.0223</td>
</tr>
<tr>
<td>APACHE II</td>
<td>0.2342</td>
<td>0.0446</td>
</tr>
<tr>
<td>SOFA</td>
<td>0.2603</td>
<td>0.0251</td>
</tr>
<tr>
<td>SAPS</td>
<td>0.3321</td>
<td>0.0038</td>
</tr>
<tr>
<td>Patients with vasoactive medication</td>
<td>0.2353</td>
<td>0.0436</td>
</tr>
<tr>
<td>Days of vasoactive medication</td>
<td>-0.08736</td>
<td>0.4656</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.256811</td>
<td>0.0560</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.2200918</td>
<td>0.1031</td>
</tr>
</tbody>
</table>

* Spearman correlation coefficient on the entire lot of patients (n=74).

Table 3. Site of infection in the studied groups

<table>
<thead>
<tr>
<th>Infection localisation</th>
<th>Sepsis group (n=40)</th>
<th>No sepsis group (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No infection</td>
<td>0</td>
<td>10 (29.41%)</td>
</tr>
<tr>
<td>Pulmonar</td>
<td>26 (65%)</td>
<td>15 (44.11%)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>4 (10%)</td>
<td>4 (11.76%)</td>
</tr>
<tr>
<td>Urinar tractus</td>
<td>2 (5%)</td>
<td>2 (5.88%)</td>
</tr>
<tr>
<td>Articular</td>
<td>0</td>
<td>1 (2.94%)</td>
</tr>
<tr>
<td>Surgical wound</td>
<td>0</td>
<td>1 (2.94%)</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>0</td>
<td>1 (2.94%)</td>
</tr>
<tr>
<td>Cutanat</td>
<td>6 (15%)</td>
<td>0</td>
</tr>
<tr>
<td>Undefined</td>
<td>2 (5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2. ROC curve for specificity and sensitivity of ANG-2 for diagnosis of sepsis

There was no statistically significant correlation between ANG-2 levels and the number of days on vasoactive medication (p=0.465). However, ANG-2 serum levels were correlated with the number of patients who required vasoactive treatment (p=0.043). A statistically significant correlation was found between ANG-2 levels and serum levels of both creatinine and urea.
Discussions

ANG-2 and diagnosis of sepsis

This study demonstrated that ANG-2 has a high sensitivity and specificity for the diagnosis of sepsis and for the prognosis of sepsis.

ANG-2 levels obtained in this study were higher than those described by other authors. Buddingh et al [11] reported an average value of 3 ng/mL ANG-2 in healthy subjects, which increased to double values in patients with severe acute pancreatitis and severe organ dysfunction, concluding that this value was highly predictive for disease severity. In the current study, the values of ANG-2 in the control group were higher (6.0 ng/mL), probably due to the fact that patients with infections, but without sepsis, were included in this group. Nevertheless, the statistically significant difference in ANG-2, between the two groups, proves that this biomarker can be useful for assessment of severity of infection in agreement with other studies [12-14].

In the current study, a cut-off value of 5.61 ng/mL was the borderline value below which the probability of infection was very low, this being similar to previously reported value of 6.0 ng/mL, reported by Buddingh et al [11] reported as representing the cutoff point above which being indicative of patients with an infection.

Several authors reported a good correlation between ANG-2 levels and pulmonary function in patients treated with high doses of interleukine-2 [15].

ANG-2, duration of stay in ICU and the requirements for vasoactive medication

Analysis of the results of the present study showed that patients without sepsis were retained intensive care units longer than those with sepsis. This apparent anomaly may be explained by the fact that sepsis patients presented a higher mortality rate, death occurring frequently in the first days after admission. A similar trend was noticed in regards to the number of days patients were maintained on vasoactive treatment.

An increase in cardiac output, related to a decrease in the left ventricular ejection fraction and hypotension has been associated with an increase of serum levels of ANG-2 [16].

ANG-2 levels and renal function.

The present study was unable to demonstrate a clinically significant correlation between ANG-2 and renal function in contradiction to the report by Tsai et al which showed ANG to be an appropriate predictor of the unfavourable development of renal failure even though in the current stage the exact pathophysiological mechanism for this effect has not been clearly identified [17].

ANG-2 levels and severity scores

Another objective of the study was to demonstrate the correlation between ANG-2 and the severity scores. We proved that ANG-2 values correlate very well with the APACHE II, SOFA and SAPS severity scores, used in the study. This supports the role ANG-2 as a biomarker for prognosis of severe sepsis, all be it that the sensitivity of this parameter for prediction of death is not high (35%). Again, this low value may reflect the fact that patients without sepsis have a high mortality rate (70.59%) due to other pathologies.
AGT-2 differentially regulates angiogenesis through tyrosine kinase-2 receptors (TIE2) and integrin signalling and several studies have demonstrated that not only the ANG-2 levels, but also TIE2 levels, may be useful in determining the prognosis of patients with severe sepsis, as the vascular modelling determined by ANG-2 is well correlated with the levels of TIE2 [18].

**CONCLUSIONS**

ANG-2 serum levels were elevated in sepsis, being well correlated with PCT values and prognostic scores. ANG-2 is both high specific and sensitive for the diagnosis of sepsis, and should be considered as a useful biomarker for the diagnosis and the prognosis of this pathology.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**