

The diagnostic and prognostic value of angiopoietins compared with C-reactive protein and procalcitonin in children with febrile neutropenia

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In this study, we aimed to determine serum angiopoietin (Ang) levels and compare them with levels of C-reactive protein (CRP) and procalcitonin (PCT). Cancer patients (aged 0-18 years) who experienced febrile neutropenia attacks were included in the study. Ang-1, Ang-2, CRP, and PCT were analyzed at admission and 2nd day. Ninety-four episodes of febrile neutropenia that developed in 62 patients were analyzed in this study. The mean age of the patients was 7.56 ± 4.8 (0.5-17) years. The patients had lymphoma (33.9%), solid tumors (48.4%), and other cancer (17.7%). The percentages of the patients with fever of unknown origin (FUO), clinically documented infection (CDI), and microbiologically documented infection (MDI) categories were 45.7%, 22.3%, and 31.9%, respectively. During the study period 11 patients were lost to follow-up. The levels of CRP, PCT and Ang-2 were significantly higher; and that of Ang-1 was significantly lower, compared to the controls.

The differentiation cannot be made between the groups by CRP and PCT levels. The level of Ang-1 was the lowest in MDI group; the level of Ang-2 and the ratio of Ang-2/Ang-1 were high in each group. Ang-1, Ang-2 and the ratio of Ang-2/Ang-1 were significantly associated with mortality. Angs correlated with the severity of infection.

Key words: angiopoietin, children, C-reactive protein, febrile neutropenia, procalcitonin.

Fever is a well-known complication in neutropenic patients with cancer.¹ During neutropenia, fever may be the first and only clinical manifestation of infection. Because of high morbidity and mortality, evaluation of the source and severity of infection must be fast in a neutropenic patient. To determine the intensity of needed treatments and to decrease the mortality rates, risk classifications were made; and these classifications were based on the measurements of C-reactive protein (CRP), procalcitonin (PCT), and interleukin 6 (IL-6) biomarkers.²⁻⁵ CRP and PCT have been reported to be guiding tools for both the prediction of infection and the arrangement of treatment; thus, they are used prevalently.⁶⁻⁸ Despite all of these biomarkers, severity of infection and risk of mortality cannot be fully

determined. Therefore, new biomarkers have been investigated.⁹⁻¹¹

Endothelial barrier disruption plays a key role in the pathogenesis of sepsis and septic shock, making it an attractive target for studies aimed to identify new therapeutic targets for sepsis.¹² Recently, the participation of VEGF, a vascular growth factor with potent microvascular permeability functions, in the pathogenesis of septic shock was demonstrated.¹³ Another important regulator of endothelial barrier function is the angiopoietins (Ang)-1 and -2, and the tyrosine kinase receptor Tie2 expressed in endothelial cells. Binding of Ang-1 to Tie2 maintains the quiescent resting state of the endothelium and reduces vascular permeability in response to inflammatory stimuli. In contrast,

Ang-2 inhibits binding of Ang-1 to Tie2, resulting in vessel destabilization.¹⁴⁻¹⁶ Circulating levels of Ang-1 and Ang-2 have been evaluated in patients with sepsis, and levels of Ang-2 have been correlated with sepsis severity in children and adults, when evaluated in patients admitted to intensive care units with established signs and symptoms of sepsis.¹⁷⁻²¹

In this study, we aimed to determine serum Ang-1, Ang-2 levels in febrile neutropenia and differences among febrile neutropenia categories. In addition, we aimed to compare Ang levels with levels of CRP and PCT.

Material and Methods

Between January 2013 and December 2014, children aged 0–18 years with cancer were included in this study during their febrile episodes of neutropenia. Characteristics of patients diagnosed with febrile neutropenia such as duration of fever and neutropenia and febrile neutropenia categories were identified, and serum levels of CRP, PCT, Ang-1, and Ang-2 were studied for two separate periods (at admission, at 48th hour of admission).

Febrile neutropenia was defined as the presence of an axillary fever $>38.5^{\circ}\text{C}$ once or $>38^{\circ}\text{C}$ twice within 4 hours in patients with an absolute neutrophil count $<500/\text{mm}^3$ or between $500\text{--}1,000/\text{mm}^3$ which is expected to decrease down to $<500/\text{mm}^3$ within 24–48 hours. Fever occurring due to blood or blood product transfusion was excluded. Febrile neutropenia episodes occurring at different times were evaluated as distinct episodes. Patient history was elicited at admission, and a physical examination was performed targeting the foci of infection. Laboratory tests were performed including complete blood count,

peripheral blood smear, serum CRP, liver (alanine aminotransferase, aspartate aminotransferase, bilirubin) and renal (urea, creatinine) functional tests, urinalysis and culture, and peripheral blood culture. In addition, catheter samples from catheterized patients were cultured. Cultures were renewed in patients with sustained high fever and bacteremia. In patients with respiratory symptoms or with positive physical examination findings, chest X-rays were obtained. In patients with suspected fungal infections, high-resolution computed chest tomography was performed. In cases with suspected typhlitis and abdominal abscess, advanced radiological imaging techniques such as abdominal ultrasound and tomographic examinations, were carried out.

Blood samples were drawn from patients at admission, and at 48th hours of fever to measure CRP, PCT, Ang-1 and Ang-2 levels. Quantitative determination of hs-CRP in serum by particle enhanced turbidimetric method using INTEGRA 800 plus system (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Ang-1 levels were measured by ELISA (ng/ml, USCN Life Science Inc, Wuhan, USA, L140801021), Ang-2 levels were measured by ELISA (ng/ml, Cusabio Biotech Co, LTD, USA, 222162891).

Febrile neutropenia was divided into three categories according to the severity of infection and febrile neutropenia guidelines: microbiologically documented infection (MDI), clinically documented infection (CDI), and fever of unknown origin (FUO).²² MDI was defined as the isolation of microorganism. CDI was considered when there was a focus of infection identified by physical examination without microbiologic documentation. FUO origin was considered when there was no

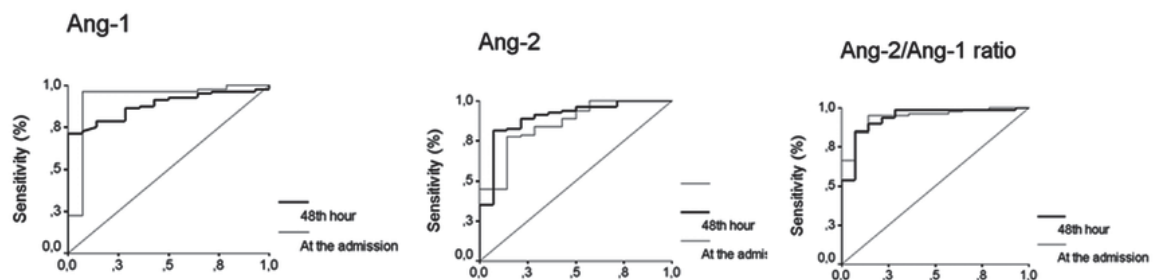


Fig. 1. Receiver operating characteristic ROC curve to determine the accuracy of angiopoietins in prediction mortality at the admission and 48th hour. (A) for Ang-1 levels, (B) for Ang-2 levels and (C) for Ang-2/Ang-1 ratio.

Table I. Demographic and Laboratory Features of Patients.

Features	Results
Age (year), mean \pm SD (range)	7.56 \pm 4.8 (0.5-17)
Gender, n (%)	
Male	34 (54.8)
Female	28 (45.2)
Primary disease, n (%)	
Non-Hodgkin lymphoma	21 (33.9)
Neuroblastoma	8 (12.9)
Osteosarcoma	6 (9.7)
Ewing sarcoma	5 (8.1)
Rhabdomyosarcoma	5 (8.1)
Wilms tumor	3 (4.8)
Germ cell tumor	3 (4.8)
Others	11 (17.7)
Stage, n (%)	
I	7 (11.3)
II	35 (56.5)
III	20 (32.3)
Febrile neutropenia category, n (%)	
FUO	43 (45.7)
CDI	21 (22.3)
MDI	30 (31.9)
Leucocyte count (/mm ³)	605 \pm 313
Absolute neutrophil count (/mm ³)	243 \pm 386
Absolute monocyte count (/mm ³)	30 \pm 15
Hemoglobin (g/dl)	8.37 \pm 1.68
Platelet count (/mm ³)	112,297 \pm 95,082

CDI: clinically documented infection, FUO: fever of unknown origin, MDI: microbiologically documented infection

clinical or microbiologic evidence of infection in a febrile episode. Infections documented both clinically and microbiologically were considered to be microbiologically documented infections. Definite sepsis included sepsis, severe sepsis, and septic shock. Sepsis was defined as systemic inflammatory response syndrome (SIRS) in the presence of or as a result of suspected or proven infection. The definition of severe sepsis is sepsis plus one of the following criteria: cardiovascular organ dysfunction, acute respiratory distress syndrome, or two or more organ dysfunctions (respiratory, renal, neurologic, hematologic, or hepatic).²³ Levels of Ang-1, Ang-2, CRP, and PCT in various categories of febrile neutropenia, and differences between levels of these parameters in these categories, were investigated.

Following approval of The Ethics Committee of Clinical Research (2013-4 TU) and obtainment of informed consent from the families who

volunteered to participate, we began our study.

Statistical Analysis

Data were analyzed using MedCalc Packet program. The data from this study were evaluated using descriptive statistical methods (mean \pm standard deviation, median, frequencies, and percentages). In the intergroup comparison of categorical variables, a chi-square test was used. Mann Whitney U-test and Kruskal Wallis test were used for comparison of independent variables, and the Wilcoxon test was used for comparison of dependent variables. Spearman's and Pearson correlation coefficient was used to assess relationships between CRP, PCT, Ang-1 and Ang-2 levels. Receiver operator characteristics (ROC) procedures were used to identify optimal cut-off values of Ang concentrations to differentiate patients in groups with FUO, CDI and MDI. A two-tailed p value less than 0.05 was considered statistically significant.

Results

A total of 94 febrile neutropenia episodes were managed in 62 patients (34 male, 28 female) with a mean age of 7.56 ± 4.8 (0.5–17) years; 50 healthy children (26 male, 24 female) with a mean age of 6.46 ± 5.26 (0.3-17) years during the study period constituted the control group. Demographics and laboratory findings of study group is shown in Table I. Median durations of neutropenia and febrile episode were 11 (3–104) and 6 (1–14) days, respectively. In CDI group 8 patients had mucositis, 3 patients had pneumonia, 2 patients had typhlitis, 2 patients had catheter related infections and 1 patient had varicella zoster infection. In MDI group, there were 29 gram negative infections (most of them *Klebsiella*, *P. aeruginosa* and *E. coli*), 22 gram positive infections (especially *coagulase negative staphylococci*) and 9 *candida* infections. Of the 94 episodes of FN, 20 (21.2%) evolved to septic shock, and 11 (11.7%) were fatal. All deaths were attributed to complications of septic shock, and occurred after a median of 11 days (range 3 to 28) from sample collection. The mean CRP, PCT, Ang-1, and Ang-1 levels and Ang-2/Ang-1 ratio at admission and at 48th hours in FUO, CDI and MDI groups and comparison between groups are shown in Table

II. A positive correlation was found between platelet count and Ang-1 levels ($r=0.77$, $p=0.001$).

When the relations between CRP, PCT levels and mortality were examined, it was determined that there was no significant difference between them (Table III). Ang-1 levels were significantly lower and Ang-2 levels were significantly higher in non-survivors, compared to survivors, both at admission and at 48th hours ($p < 0.001$; Table III). Threshold was determined to the usability of determining the mortality of the all parameters. ROC analyses ad sensitivity, specificity, positive and negative predictive values of Ang-1, Ang-2 and Ang-2/Ang-1 ratio at specific values are presented in Figure 1 and Table IV.

Discussion

Post chemotherapy FN in patients with hematological malignancies is a condition that carries a high risk of sepsis complications with mortality rates as high as 21%, usually preceded by septic shock.²⁴ Patients with FN are a heterogeneous group in terms of risks of complications and mortality, and the identification of parameters capable to accurately identify high risk patients is one of the great PCT have been recently proved to be

Table II. CRP, Procalcitonin, Angiopoetin-1, Angiopoetin-2, Angiopoetin-2/Angiopoetin-1 Test Results in Group of Febrile Neutropenia.

	CRP (mg/L)	PCT (ng/ml)	Ang-1 (ng/ml)	Ang-2 (ng/ml)	Ang-2/Ang-1 ratio
FUO					
At the admission	46.19±61.24	2.39±2.22	1.33±0.49	3.52±3.02	3.62±5.49
48 th hour	65.64±70.49	1.69±0.96	1.79±4.02	1.97±2.14	5.31±10.57
CDI					
At the admission	74.24±55.04	2.58±1.89	1.14±0.49	6.59±2.52	8.61±8.79
48 th hour	92.20±49.62	2.44±7.38	0.93±0.54	5.12±2.13	8.24±6.91
MDI					
At the admission	101.84±72.90	10.51±7.87	0.64±0.40	12.63±3.99	31.48±30.96
48 th hour	131.03±81.94	8.95±11.46	0.40±0.29	10.67±4.01	51.48±51.93
p ¹	0.04	0.063	0.313	< 0.0001	< 0.0001
p ²	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
p ³	0.063	< 0.0001	< 0.001	< 0.0001	< 0.0001
p ⁴	0.01	0.076	0.488	< 0.0001	< 0.001
p ⁵	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
p ⁶	0.072	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Ang-1: angiopoietin-1, Ang-2: angiopoietin-2, CDI: clinically documented infection, CRP: C-reactive protein, MDI: microbiologically documented infection, FUO: fever of unknown origin, PCT: procalcitonin, p¹: FUO vs CDI at admission, p²: FUO vs MDI at admission, p³: CDI vs MDI at admission, p⁴: FUO vs CDI at 48th hour, p⁵: FUO vs MDI at 48th hour, p⁶: CDI vs MDI at 48th hour

Table III. CRP, Procalcitonin, Angiopoetin-1, Angiopoetin-2, Angiopoetin-2/Angiopoetin-1 Test Results in Survivor and Non-survivor Patients.

	Survivors (n=52)	Non-survivors (n=11)	p value
CRP (mg/L) at the admission	67.97±69.92	83.08±54.43	0.121
CRP (mg/L) at the 48 th hour	86.98±72.13	123.66±88.23	0.104
PCT (ng/ml) at the admission	4.79±6.79	6.62±5.25	0.066
PCT (ng/ml) at the 48 th hour	3.93±8.18	6.76±11.47	0.057
Ang-1 (ng/ml) at the admission	1.18±0.50	0.41±0.33	< 0.0001
Ang-1 (ng/ml) at the 48 th hour	1.32±2.99	0.22±0.13	< 0.0001
Ang-2 (ng/ml) at the admission	6.00±4.23	13.44±5.38	< 0.0001
Ang-2 (ng/ml) at the 48 th hour	4.29±3.65	12.03±5.01	< 0.0001
Ang-2/Ang-1 ratio at the admission	7.87±10.26	46.49±38.15	< 0.0001
Ang-2/Ang-1 ratio at the 48 th hour	10.30±20.08	80.09±52.50	< 0.0001

Ang: angiopoetin, CRP: C-reactive protein, PCT: procalcitonin

useful tools for this purpose.²⁵ However, it is generally acknowledged that these parameters are more useful to identify patients at low risk for complications. There are limited studies evaluating importance of CRP and proinflammatory cytokine levels in detecting bacterial infection and clinical progress during febrile neutropenia attack.

Santolaya et al.²⁶ showed a CRP value of >40 mg/L discriminated children with a demonstrated bacterial infection (sensitivity 100%, specificity 76.6%) from children with febrile neutropenia. They also showed that children with an unfavorable outcome had persistently high levels of serum CRP. Rintala et al.²⁷ found that CRP levels were significantly higher when the blood culture was positive and CRP levels were significantly higher when the infection focus was identified. They concluded that the serial measurement of CRP is a reliable and readily available means for differentiating between bacterial infections and other causes of fever in patients with hematological malignancies, during neutropenia and after cytotoxic treatment. Mian et al.²⁸ showed that CRP >100 mg/L had a sensitivity of 88% in predicting high-risk FN.

Sakr el al.²⁹ reviewed 30 articles which investigated the PCT levels in FN. They found that PCT seems to be able to discriminate fever due to systemic types of infection from non-infectious etiologies. They also found that PCT might be useful in outcome prediction in patients with FN but it was not superior

to CRP concentration. Engel et al.³⁰ detected an increase in PCT levels in the first 32 hours of fever in patients with clinically or microbiologically proven infection. They also found that PCT levels were lower in patients with fever of unknown origin when compared with proven infection. Karakurt DG et al.² did not find IL-6, IL-8, sTNFR1I, sIL-2R, CRP, and PCT levels as significant predictors of serious infectious complication in pediatric cancer patients' admission for febrile neutropenia. The concentration of lipopolysaccharide-binding protein (LBP), PCT, interleukin-6, and CRP was determined for 90 episodes of FN by Kitanovski et al.³¹ The concentrations of all biomarkers were significantly higher in patients with bacteremia/clinical sepsis than non-bacteremia/clinical sepsis episodes on admission and day 2. The diagnostic accuracy of PCT did not significantly different from CRP. A recent meta-analysis showed that the PCT test has higher diagnostic value than CRP or IL-6 for the detection of bacterial infection in patients with FN. In that study, data suggested that PCT is a more specific than sensitivity and helps to confirm bacterial infections rather than rule them out.³²

Our results showed that a lowest CRP level was found in FUO group. According to CRP levels we could not distinguished the CDI and MDI group in this study both at the admission and at 48th hours. In our study CRP levels were not statistically different in survived and non-survived patients. CRP was not a useful

marker for detecting the mortality according to our study. These results are similar to the literature.^{10,11} We could not distinguish FUO and CDI according to PCT levels. The highest level of PCT was found in MDI group in our study. These results showed that PCT is more useful, compared to CRP, to discriminate the MDI group. Our results also showed that PCT levels (both at admission and at the 48th hour) were helpful to predict bacteremia. These results are similar to the literature.^{10,11} However, PCT levels were not helpful to predict the mortality.

In the developed vasculature, Ang-1 protects against vascular leakage, whereas Ang-2 promotes increased vascular permeability. Ang-1 and Ang-2 appear an agonist–antagonist effect to pair mediating capillary endothelium leakage (i.e., Ang-2/Ang-1). The higher the Ang-1/Ang-2 ratio is, the more severe the capillary endothelial damage. In addition, Ang-1 has been proposed to be necessary for the maintenance of vascular barrier function via its interaction with Tie-2 in blood vessels. As a default pathway, constitutive Ang-1–Tie-2 interactions promote endothelial cell migration, proliferation, survival, and differentiation and regulate endothelial barrier integrity and stability.^{11,33} A lower Ang-1/Tie-2 ratio indicates a stronger defense against capillary endothelial damage. Taken together, the increase in the Ang-2/Ang-1 ratio and the decrease in the Ang-1/Tie-2 ratio demonstrate that destruction of the capillary endothelium is greater than the stability in the deterioration of sepsis. Thus, high admission serum Ang-2/Ang-1 ratio and

low admission serum Ang-1/Tie-2 ratio identify the severity of sepsis in patients at the onset of early sepsis in the emergency department.³⁴

Increased levels of Ang-2 levels have been demonstrated in adult and pediatric patients with sepsis in intensive care units (ICU) and these levels correlated with sepsis severity.^{17,18,35} Serum obtained from patients with sepsis has been shown to disrupt endothelial architecture, an effect that correlates with Ang-2 levels, is reversed by Ang-1.³⁶

Kumpers et al.¹⁸, studying a population of 43 medical ICU patients demonstrated that an Ang-2 level at the time of ICU admission was a strong predictor of mortality. Ang-1 levels were also evaluated in some of these studies, but so far studies have failed to demonstrate a relationship between Ang-1 and sepsis outcomes.

To our knowledge, there were 2 studies about FN and angiopoietins in adult population.^{10,11} Our study is the first one with neutropenic fever attacks in childhood. Our results suggest that the decrease in the level of Ang-1 occurs to protect the damage of the endothelial barrier in MDI group. The results at 48th hour are similar to that at the admission: the level of Ang-2 is significantly higher in MDI and CDI group, compared to FUO group. We may think that the increase in the level of Ang-2 is related to damage in the endothelial barrier. Moreover, it has a role as a distinctive feature among FUO, CDI and MDI groups and has a positive correlation with the severity of the infection and this could be a distinctive parameter for

Table IV. Specificity, Sensitivity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Diagnostic Performance of Angiopoietin (Ang)-1, Ang-2 Concentrations and Ang-2/Ang-1 Ratio According to Cutoff Values at the Admission and 48th Hour for Predicting Mortality.

	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	Diagnostic Performance
At the admission					
Ang-1 (≤ 0.452 ng/ml)	96.2	92.6	81.2	98.7	0.920
Ang-2 (> 9.168 ng/ml)	77.5	85.7	40.0	96.9	0.854
Ang-2/Ang-1 (> 21.71)	95.0	85.8	75.0	97.4	0.942
48 th hour					
Ang-1 (≤ 0.436 ng/ml)	71.2	100.0	37.8	100.0	0.881
Ang-2 (> 7.675 ng/ml)	81.2	92.9	46.4	98.5	0.896
Ang-2/Ang-1 (> 17.32)	85.0	92.9	52.0	98.6	0.937

these groups but needs to be supported by other studies.

In adult studies, only ratio of Ang-2 to Ang-1 were found predictive parameters for mortality.^{10,11} Contrary to the data obtained from studies in adults, both Ang-1, Ang-2 and the ratio of Ang-2 to Ang-1 had determinant effects on mortality in our study. In the current study, the sensitivity, specificity, positive and negative predictive value of Ang-1, Ang-2 and the ratio of Ang-2 to Ang-1 were comparable. We assume that the difference of the data in adults is due to the immaturity of the endothelial barrier in childhood. But as it is the first study about FN in childhood, the results have to be supported by the further studies.

In conclusion, our study suggests that Ang-1, Ang-2, and Ang-2/Ang-1 ratio are biomarkers for 28-day mortality in childhood FN.

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