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Original Article

The role of proangiogenic cytokines in predicting sepsis in febrile neutropenic children with cancer

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ABSTRACT

Background. We assessed the relationship between sepsis occurrence and the serum levels of angiopoietin (Ang-1, Ang-2), vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) in pediatric patients with cancer-related febrile neutropenia.

Methods. Fifty-two children with malignant tumors who experienced 86 episodes of febrile neutropenia (FN) were examined between June 2016 and June 2018. Each FN episode was considered a separate event and the total number of FNs were recorded (86 FN episodes = FN group). The control group consisted of 21 healthy children. Ang-1, Ang-2, VEGF-A and sFlt-1 were measured at the baseline and 48th hour of each FN episode –alongside routine characterization of inflammation (C-reactive protein; white blood cell and absolute neutrophil count).

Results. Among the episodes, 29 (34.5%) developed sepsis while 57 were classified as non-complicated FN. The baseline values of patients and controls were significantly different for Ang-1, Ang-2, VEGF and sFlt-1 values (all, $p < 0.05$). In the subgroup with sepsis, Ang-2 values were higher than in the subgroup without sepsis ($p = 0.017$). In predicting sepsis, Ang-2 had 60.7% sensitivity and 66.7% specificity at the 74.6 cut-off value (AUC: 0.662 [95%CI: 0.541 – 0.783], $p = 0.022$), Ang-2 / Ang-1 ratio had 65.5% sensitivity and 60.0% specificity at the 0.405 cut-off value (AUC: 0.633 [95%CI: 0.513 – 0.753], $p = 0.046$).

Conclusions. Our results reveal that Ang-2 and Ang-2/Ang-1 were higher in the sepsis group and Ang-2 might be a biomarker to indicate the risk of sepsis in patients with FN and/or cancer.

Key words: sepsis, children, febrile neutropenia, angiopoietin, vascular endothelial growth factor, soluble fms-like tyrosine kinase.

Although there has been a significant improvement in the course of childhood cancers in recent years, infections remain as the primary cause of death and morbidity.[1] Neutropenia-associated fever develops during chemotherapy in approximately 80% of hematologic malignancies and 10-50% of solid tumors.[2] In the absence of fever, but in the presence of findings indicating focal or systemic infection, neutropenic patients are treated within the scope of febrile neutropenia (FN). FN is also associated with significant morbidity, mortality, a decrease in, and postponement of chemotherapy and the cost of treatment.[2] Several studies have aimed to make empirical therapy feasible by defining risk factors for serious infections and sepsis.[3] Although risk classifications exist for adults, there are no validated risk stratification schemas for the pediatric population.

Neutrophils, macrophages and endothelial cells play a role in early oxidative stress occurring during sepsis and fight against the pathogen as the first defense mechanism of the immune

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system.[4] In the human body, the endothelium with a surface area of about 1000 m^2 is a very dynamic organ. Endothelium forms a surface between blood and tissue and also has an important role in regulating vascular tone, coagulation and inflammation response. The endothelium, which is exposed to the direct effect of microorganisms and products during sepsis and is highly activated, becomes self-injurious after a while. Vascular endothelial growth factor (VEGF) triggers endothelial cell proliferation, migration, and differentiation. The significance of VEGF extends to its crucial role in both childhood and adulthood for the processes of vasculogenesis and angiogenesis [5]

The best-known members of angiopoietins (Ang), another family of growth factors affecting endothelial barrier function, are Ang-1 and Ang-2, which exert their influence via Tie receptors.[6] The biological process of angiogenesis is closely regulated by many factors including Ang-1, Ang-2, VEGF and soluble fms-like tyrosine kinase-1 (sFlt-1). The barrier function of endothelium is bolstered by sFlt-1 and Ang-1; whereas, VEGF-A and Ang-2 act to disrupt cellular junctions.[7] Owing to their direct involvement in the endothelium, researchers have explored these proteins for their association with the sepsis process, with promising data being reported for different groups of patients.[8-13]

We aimed to measure serum levels of Ang-1, Ang-2, VEGF, sFlt-1, and calculate Ang-2/Ang-1 ratio as a marker of capillary endothelial injury[12] in pediatric cancer patients with febrile neutropenia. These results were then used to assess whether these parameters could be utilized to predict the risk of sepsis in this population.

Material and Methods

Study design

The study has been conducted in accordance with the principles of the Helsinki Declaration and approved by the ethics committee of Ankara Oncology Hospital (Date: 03.05.2016/ No.20033663). Written informed consent was obtained from the parents or legal guardians.

We prospectively evaluated 52 cancer patients aged 0-18 years with FN treated between June 2016 and June 2018. Each FN episode was considered a separate event, and the total number of FNs was recorded (86 FN episodes). The control group comprised 21 healthy children.

Inclusion criteria

1) Fever ≥ 38°C lasting for over one-hour or 38.3° measured once, 2) Chemotherapy-induced severe neutropenia (absolute count < 500/mm^3 or anticipated to drop to this level within 24-48 hours from 500-1000/mm^3).

Febrile neutropenia protocol and sepsis definitions

Blood cultures were drawn in accordance with FN protocols (central venous catheter lumens with concurrent peripheral cultures) at fever onset. Urinalysis and urine culture were acquired with clean-catch, midstream specimens. Other laboratory tests were performed including a complete blood count, peripheral blood smear, serum C-reactive protein (CRP), liver (alanine aminotransferase, aspartate aminotransferase, bilirubin) and renal (urea, creatinine) functional tests. Standardized chest X-ray protocols were adhered to when managing patients with relevant symptomatology.[14] Empirical monotherapy was initiated promptly in all patients.

Sepsis was characterized by the manifestation of two (or more) of the following: 1) Temperature > 38.5°C or < 36°C, 2) Tachypnea (adjusted for age) unrelated to neuromuscular disease or anesthesia, 3) Tachycardia or bradycardia (adjusted for age), along with a clinically evident source of infection or a microbiologically documented infection.[15] In accordance with this classification, we categorized febrile neutropenia (FN) episodes into two groups: (i) Non-complicated FN and (ii) Sepsis.

Outcome parameters

Blood specimens were obtained at fever onset (baseline) and 48 hours later. Following routine centrifugation for serum separation, samples were preserved at -80°C until analysis. The processing of samples was performed by a single investigator who was unaware of the patients' outcomes.

Laboratory analyses

The quantification of targeted analytes were performed with enzyme-linked immunosorbent assays (ELISA). A ELx 800 microplate reader (BioTek Instruments, Vermont, USA) and ELx50 microplate strip washer (BioTek Instruments, Vermont, USA) were used. The assays were performed according to ELISA kit manufacturer's instructions. Measurements exceeding the linear range underwent repeat analysis with appropriate dilution. The RayBio® Human ANGPT1 ELISA kit (Georgia, USA) and RayBio® Human ANGPT2 ELISA kit (Georgia, USA) were used for the measurement of ANGPT-1 and ANGPT-2.

The minimum detectable concentrations for Human ANGPT1 and Human ANGPT2 were established at 30 pg/ml and 10 pg/ml, respectively. The intraassay coefficient of variation (CV) was below 10%, and the interassay CV was below 12% for both ELISA kits.

For the Human VEGF-A ELISA kit (eBioscience Thermo Fisher, California San Diego, USA), the analytical sensitivity was 7.9 pg/mL, and the assay range spanned from 15.6 to 1,000 pg/ml. The interassay CV was 4.3%, while the intraassay CV was 6.2%.

The VEGF Receptor 1 Monoclonal Antibody (Hu VEGF-A) ELISA VEGF- A ELISA (e Bioscience Thermo Fisher, California San Diego, USA) kit analytical sensitivity was 0.03 pg/mL and the assay range was between 0.16-10 pg/mL. The interassay CV was 5.1 % whereas intraassay CV was 5,5 %.

Statistical analysis

The SPSS v20 software was used for analysis. The Kolmogorov-Smirnov test was employed to assess the normal distribution suitability of the data. Continuous variables were presented as median (minimum – maximum), while categorical variables were expressed as frequency (percentage). The Mann-Whitney rank sum test was used to scrutinize differences in continuous variables between patients and healthy controls. Additionally, the Wilcoxon test was applied to compare two dependent groups (baseline vs. 48th hour). Binary logistic regression analysis was carried out to investigate independent risk factors influencing sepsis. Determination of optimal cut-off values for biomarker concentrations was conducted through receiver operator characteristics (ROC) analysis and the Youden Index. The statistical evaluation was performed at a 95% confidence level, and significance was attributed if the p-value was less than 0.05.

Results

The study encompassed 52 participants aged between 0 and 18 years, comprising 28 males and 24 females, along with 21 controls. The age and sex distribution of the groups were similar ($p > 0.05$).

The most common diagnoses were acute lymphoblastic leukemia (22%), osteosarcoma (22%), and Ewing's sarcoma (22%). The median duration of neutropenia was 6 days (min–max: 3-30), and absolute neutrophil count of the whole group was 55/mm^3 (min–max: 10-900). The groups were similar for age, sex, remission, diagnosis, neutrophil count ($p > 0.05$). Patients with sepsis had significantly longer duration of neutropenia and fever ($p < 0.001$) (Table I).

Of the 86 FN episodes, 29 (34%) were complicated with sepsis. A microbiological agent was isolated in 11 (13%) episodes (6 methicillin resistant *Staphylococcus aureus*, 3 *Escherichia coli*, 1 *Enterobacter spp.*, 1 *Candida spp*). Eight patients had pneumonia, 6 had mucositis,

Table I. Characteristics of FN episodes with and without sepsis.

Features	Sepsis (-) (n:57)	Sepsis (+) (n:29)	Total (n=86)	*P*-value
Age (years)				
Range	0.7-17	0.7-18	0.7-18	0.374
Median	13	14	13	
Sex				
Male	27 (47%)	14 (48%)	41 (48%)	0.937
Female	30 (53%)	15 (52%)	45 (52%)	
Primary diagnosis				
Ewing sarcoma	17 (30%)	2 (7%)	19 (22%)	0.102
ALL	14 (25%)	5 (17%)	19 (22%)	
Osteosarcoma	11 (19%)	8 (28%)	19 (22%)	
AML	2 (4%)	6 (21%)	8 (10%)	
RMS	7 (12%)	1 (3%)	8 (10%)	
NHL	1 (2%)	5 (17%)	6 (7%)	
Others	5 (8%)	2 (7%)	7 (7%)	
Disease status				
AD	17 (30%)	13 (45%)	30 (35%)	0.645
PR	11 (20%)	3 (10%)	14 (16%)	
CR	23 (40%)	7 (24%)	30 (35%)	
R/RD	6 (10%)	6 (21%)	12 (14%)	
Neutrophil count (cells/mm3)				
Range	10-900	10-690	10-900	0.165
Median	60	50	55	
Duration of neutropenia (days)				
Range	3-30	3-30	3-30	<0.001
Median	6	8	6	
Days with fever				
Range	1-8	2-15	1-15	<0.001
Median	2	5	3	

*Data are given as median (min- max) for continuous variables and as frequency (percentage) for categorical variables
AD: active disease, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, CR: complete remission, RMS: rhabdomyosarcoma, NHL: non-Hodgkin lymphoma, PR: partial remission, R/RD: relapse/resistant disease

3 had anal abscess, 2 had cellulitis and 1 had catheter insertion-site infection. Additionally, 7 patients had catheter-related infection, one with pneumonia and another with urinary tract infection. None of the cases were fatal. Of the 86 FN episodes, 35% were recorded during active disease, 35% in complete remission, 16% in partial remission and 14% during relapsed/resistant disease. Demographic, clinical and laboratory data were similar in patients with sepsis or non-complicated FN.

Ang-1 and VEGF were significantly higher in controls; whereas, Ang-2, Ang-2/Ang-1 and sFlt-1 were higher in the FN group ($p < 0.05$). Baseline and 48th hour Ang-1, Ang-2/Ang-1 and VEGF values were similar in patients with FN ($p > 0.05$). However, Ang-2 and sFlt-1 values demonstrated a significant change, the former was higher at 48 hours while the latter was lower ($p < 0.05$) (Table II). Baseline Ang-2 and Ang-2/Ang-1 values in the sepsis subgroup were higher compared to the non-sepsis group

Table II. Baseline and 48th hour measurements of control and FN groups.

	Control (n=21)	FN (NOE: 86) Baseline	FN (NOE: 86) 48th hour	*p*1	*p*2
Ang-1 (ng/ml)	5614.04 (1501.03-13305.35)	303.51 (11.57 – 14452,17)	370.37 (32.41 – 14913.04)	< 0.001	0.370
Ang-2 (ng/ml)	50.79 (18.11 – 2013.70)	89.41 (15.64 – 1111.11)	165.26 (22.3 – 2897.40)	0.022	< 0.001
Ang 2 / Ang 1	0.01 (0.00 – 0.35)	0.36 (0.00 – 11.28)	0.55 (0.00 – 10.48)	<0.001	0.084
VEGF (pg/mL)	211.67 (108.44 – 1000.0)	55.33 (0.00 – 1000.00)	40.35 (0.00 – 1000.0)	< 0.001	0.052
sFlt-1 (ng/ml)	940.00 (780.00 – 1000.00)	1150.00 (750.00 – 3980.00)	1010.00 (720.00 – 4210.00)	< 0.001	0.004

Data are given as median (min – max)
Ang: Angiopoetin, FN: febrile neutropenia, IQR: interquartile range, NOE: number of episodes, sFlt-1: soluble fms -like tyrosine kinase-1, p1: Control vs FN baseline, p2: FN baseline vs 48th hour, VEGF: vascular endothelial growth factor

Table III. Baseline and 48th hour measurements according to the presence of sepsis.

	Baseline			48th hour		
	Sepsis (-) n:57	Sepsis (+) n:29	*p*1	Sepsis (-) n:57	Sepsis (+) n:29	*p*2
Ang-1 (ng/ml)	417.35 (11.5 – 14452.17))	197.75 (12.15 – 1983.64)	0.191	448.06 (32.41 – 15913.04)	201.04 (41.66 – 14452.17)	0.154
Ang-2 (ng/ml)	75.13 (15.64 – 831.17)	145.82 (23.05 – 1111.11)	0.017	154.39 (23.05 – 902.15)	172.21 (22.37 – 2897.40)	0.964
Ang 2/ Ang 1	0.21 (0.00 – 11.28)	0.58 (0.03 – 9.49)	0.046	0.48 (0.00 – 10.48)	0.70 (0.02 – 9.60)	0.344
VEGF (pg/ml)	58.13 (0.00 – 1000.00)	44.72 (9.88 – 1000.00)	0.504	51.27 (0.00 – 1000.00)	37.23 (0.00 – 821.28)	0.279
sFlt-1 (ng/mL)	1120.00 (750.00 – 3980.00)	1380.00 (770.00 – 3130.00)	0.294	1010.00 (720.00 – 4210.00)	1020.00 (740.00 – 3630.00)	0.631

Data are given as median (min – max)
Ang: Angiopoetin, sFlt-1: soluble fms -like tyrosine kinase-1, VEGF: vascular endothelial growth factor

($p < 0.05$). No significant difference was found in Ang-1, VEGF and sFlt-1 values ($p > 0.05$) The 48th hour values for all parameters were similar in the sepsis and non-sepsis groups ($p > 0.05$) (Table III).

In predicting sepsis, Ang-2 had 60.7% sensitivity and 66.7% specificity at the 74.6 cut-off value (AUC: 0.622 [95% CI: 0.541 – 0.783], $p = 0.022$). The Ang-2 / Ang-1 level had 65.5% sensitivity and 60.0% specificity at the 0.405 cut-off value (AUC: 0.633 [95% CI: 0.513 – 0.753], $p = 0.046$) (Fig. 1).

Discussion

Patients with hematological malignancies are prone to sepsis and sepsis complications due to intensive chemotherapy.[16] Studies have shown that in order to minimize mortality and morbidity in sepsis, it is important to distinguish those with the highest risk of complications and to initiate early and prompt treatment.[17] However, there are no reliable biomarkers that facilitate the prediction of sepsis development in patients with FN.[16,18]

VEGF-A, sFlt-1, Ang-1, and Ang-2 are crucial for angiogenesis. Although each factor has nuanced roles in the grand processes that impact angiogenesis, it is well established that VEGF-A and Ang-2 destabilize cell junctions, while sFlt-1 and Ang-1 carry out re-stabilization of the endothelial barrier. As a result, endothelial functionality retains its capability to carry out its all-important duty in vessel propagation and destruction. It is evident that, through their concerted and opposing effects, these four factors contribute to the critical process of forming, re-forming, and destroying vessels, which continues throughout life.[12]

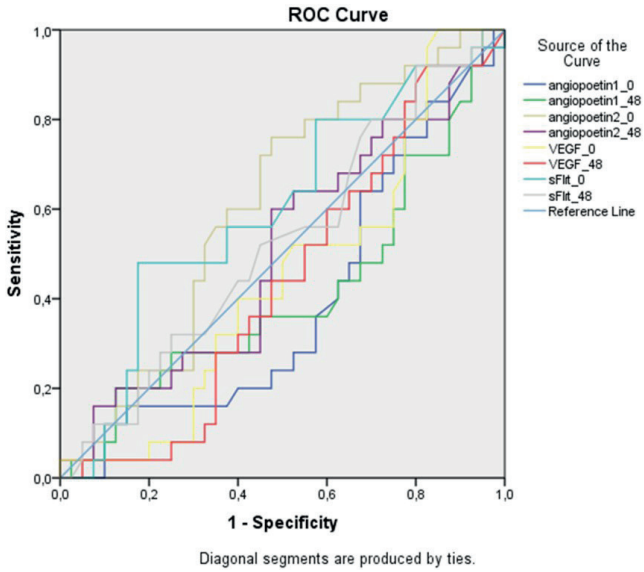

Fig. 1. Receiver operating characteristic curves of serum biomarkers at baseline (0) and at the 48th hour (48) with regards to the presence of sepsis. sFlt-1: soluble fms-like tyrosine kinase-1; VEGF: vascular endothelial growth factor.

Owing to their impact on vessels and the endothelium, we attempted to investigate whether Ang-1, Ang-2, VEGF or sFlt-1 could be utilized to predict the risk of sepsis among pediatric patients with FN. Our study showed that Ang-2 and Ang-2/ Ang-1 values were higher at baseline measurement in FN patients with sepsis. However, since the ROC analysis revealed low sensitivity and specificity, we can conclude that these parameters are weakly associated with predicting sepsis.

There are five members in the VEGF-related family of molecules, but VEGF-A has been demonstrated to be prominent in the context of sepsis.[11] Increased levels of circulating VEGF are detected in meningitis and shock.[19] It has been observed that VEGF levels increase in many conditions that cause disruption of endothelial integrity, especially sepsis.[20] In addition, VEGF level is a discriminatory factor that can predict mortality in patients admitted to the intensive care unit due to sepsis.[21,22] In an investigation involving 42 hematological patients experiencing FN, individuals with sepsis exhibited elevated VEGF compared to the non-sepsis group.[23] In a substantial clinical study by Karlsson et al., the established correlation between VEGF level and severe sepsis was once again validated. Interestingly, the authors also reported that patients progressing to shock had a substantial decrease in VEGF, suggesting a predictive capability for endothelial dysfunction.[21] We did not detect a disparity in VEGF levels between our groups (with and without sepsis) at neither baseline nor 48th hour measurement. Contrary to the literature, these results question whether VEGF is a useful biomarker for sepsis and its severity. It is of note that 37% of our cases were recently diagnosed with acute leukemia and were receiving induction therapy, potentially indicating that VEGF levels may be misleading to assess sepsis in this group of subjects. This interpretation may indeed be true, as reports have shown decreased levels of VEGF in acute leukemias, both at diagnosis and during induction therapy.[24]

sFlt-1 acts as a receptor for both VEGF and placental growth factor. In mouse models, sFlt-1 administration improves outcomes in sepsis by regulating inflammation.[19] Some studies report promising data for sFlt-1, even suggesting it

to be a reliable measure of sepsis severity.[25,26] Recombinant sFlt-1 has been shown to reduce inflammatory cytokine levels and protect mice from VEGF-A-mediated sepsis.[27] In our study, sFlt-1 levels were similar in the sepsis and non-sepsis groups, and also, there was no difference in the FN vs. control comparison. We believe that our results may be related to profound neutropenia and thrombocytopenia, as neutrophils and platelets are the main sources of these receptors. It is also known that circulating levels of proangiogenic cytokines, including VEGF, increase in both adult and pediatric malignancies. In addition, we cannot predict whether the underlying type of cancer and remission status affect basal blood levels of these mediators. For various reasons, such as these, there seems to be insufficient evidence for VEGF and sFlt-1 to be a sepsis biomarker in FN.

Ang-1 has gained notable renown as a potential measure of sepsis severity in the early phase of disease. Mankhambo et al.[28] demonstrated that decreased Ang-1 and elevated Ang-2 were linked to unfavorable outcomes in 293 children diagnosed with severe bacterial infections. In their multicenter study involving 70 patients, Ricciuto et al.[9] reported similar results, strengthening the prior interpretation. In studies focusing on other medical fields, Ang-2 has been associated with endothelial cell apoptosis, inflammation, vascular dysfunction and lung epithelial damage, all of which can be a result of sepsis.[29-31] As can be understood from most of the literature, besides vascular dysfunction in sepsis, angiopoietins can contribute to the pathophysiology of sepsis. In the present study, baseline Ang-2 values were significantly different among patients who ultimately developed or did not develop sepsis. In the subgroup with sepsis, Ang-2 values were higher than those of the subgroup without sepsis at admission. However, the baseline Ang-2 value at 74.6 cutoff points fails to predict sepsis strongly (60.7% sensitivity and 66.7% specificity). This finding was inconsistent with previous studies demonstrating that baseline Ang-2 can be utilized to assess sepsis risk.[9,32-34] In some of the mentioned studies, Ang-2 was shown to be high in the sepsis group at admission and increased gradually at the 48th hour. We did not detect any significant difference for Ang-2 in the comparison of baseline to 48th hour results among sepsis patients. Nonetheless, Ang-2 values were higher at admission and continued to demonstrate an increasing trend. Taken together, it appears that Ang-2 levels change during the acute phase of sepsis. The absence of mortality in our study may have limited the alteration, and therefore, we may have been unable to observe the previously-reported increase in Ang-2 levels. Additionally, we cannot comment on the association between Ang-2 and mortality.

In our group of patients, we detected a significant distinction between the control and FN groups with respect to baseline values of Ang-1, Ang-2, Ang-2/Ang-1 ratio, VEGF, and sFlt-1. Specifically, Ang-1 and VEGF levels were observed to be higher in the control group, whereas Ang-2 and sFlt-1 values were elevated in the patient group. Within the patient group, no significant variance was detected in baseline versus 48th-hour comparisons of Ang-1, Ang-2/Ang-1 ratio, and VEGF values. However, Ang-2 and sFlt-1 values demonstrated significant differences, with elevated Ang-2 and decreased sFlt-1 values at the 48th hour.

The small sample size was the most important limiting factor in our study. The second limitation was that the experience belonged to a single center and the third was that the patient group and the healthy groups were distinguished by more than one factor, which would ideally have been FN. The control group was comprised of healthy children without cancer, which could bias the results. Therefore, in the context of predictive performance, the inclusion of another group with cancer but without FN or sepsis could prove crucial for a comprehensive comparison of Ang-1 and Ang-2 levels in future studies.

In FN patients with sepsis, elevated levels of Ang-2 and the Ang-2/Ang-1 ratio were observed

at baseline. Notably, the baseline Ang-2 value was identified as a factor associated with an augmented risk of sepsis in individuals with cancer experiencing FN. Consequently, Ang-2 emerges as a potential biomarker indicative of the risk of sepsis in this clinical context. However, the comprehensive understanding of how these studied biomarkers interact with other inflammatory mediators, particularly additional vascular mediators, necessitates further exploration through larger-scale studies.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of Ankara Oncology Hospital (Date 03.05.2016 / No.20033663) Written informed consent was obtained from the parents or legal guardians.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SÇ, NS; data collection: SÇ, NS; analysis and interpretation of results: ÇS, SÇ, NS, İEİ; draft manuscript preparation: SÇ, NS, ÇS, İEİ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Stone WL, Klopfenstein KJ, Hajianpour MJ, Popescu MI, Cook CM, Krishnan K. Childhood cancers and systems medicine. Front Biosci (Landmark Ed) 2017; 22: 1148-1161. https://doi.org/10.2741/4538
2. Celkan T, Koç BŞ. Approach to the patient with neutropenia in childhood. Turk Pediatri Ars 2015; 50: 136-144. https://doi.org/10.5152/TurkPediatriArs.2015.2295
3. White L, Ybarra M. Neutropenic fever. Hematol Oncol Clin North Am 2017; 31: 981-993. https://doi.org/10.1016/j.hoc.2017.08.004
4. Mantzarlis K, Tsolaki V, Zakynthinos E. Role of oxidative stress and mitochondrial dysfunction in sepsis and potential therapies. Oxid Med Cell Longev 2017; 2017: 5985209. https://doi.org/10.1155/2017/5985209
5. Whitney JE, Silverman M, Norton JS, Bachur RG, Melendez E. Vascular endothelial growth factor and soluble vascular endothelial growth factor receptor as novel biomarkers for poor outcomes in children with severe sepsis and septic shock. Pediatr Emerg Care 2020; 36: e715-e719. https://doi.org/10.1097/PEC.0000000000001638
6. Siner JM. A tale of two ligands: angiopoietins, the endothelium, and outcomes. Crit Care 2013; 17: 1007. https://doi.org/10.1186/cc13066
7. van der Flier M, van Leeuwen HJ, van Kessel KP, Kimpen JL, Hoepelman AI, Geelen SP. Plasma vascular endothelial growth factor in severe sepsis. Shock 2005; 23: 35-38. https://doi.org/10.1097/01.shk.0000150728.91155.41
8. Siner JM, Bhandari V, Engle KM, Elias JA, Siegel MD. Elevated serum angiopoietin 2 levels are associated with increased mortality in sepsis. Shock 2009; 31: 348-353. https://doi.org/10.1097/SHK.0b013e318188bd06
9. Ricciuto DR, dos Santos CC, Hawkes M, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. Crit Care Med 2011; 39: 702-710. https://doi.org/10.1097/CCM.0b013e318206d285
10. Alves BE, Montalvao SAL, Aranha FJP, et al. Time-course of sFlt-1 and VEGF-A release in neutropenic patients with sepsis and septic shock: a prospective study. J Transl Med 2011; 9: 23. https://doi.org/10.1186/1479-5876-9-23
11. Paulus P, Jennewein C, Zacharowski K. Biomarkers of endothelial dysfunction: can they help us deciphering systemic inflammation and sepsis? Biomarkers 2011; 16 Suppl 1: S11-S21. https://doi.org/10.3109/1354750X.2011.587893
12. Luz Fiusa MM, Costa-Lima C, de Souza GR, et al. A high angiopoietin-2/angiopoietin-1 ratio is associated with a high risk of septic shock in patients with febrile neutropenia. Crit Care 2013; 17: R169. https://doi.org/10.1186/cc12848

13. Fang Y, Li C, Shao R, Yu H, Zhang Q. The role of biomarkers of endothelial activation in predicting morbidity and mortality in patients with severe sepsis and septic shock in intensive care: a prospective observational study. Thromb Res 2018; 171: 149-154. https://doi.org/10.1016/j.thromres.2018.09.059

14. Lehrnbecher T, Robinson P, Fisher B, et al. Guideline for the management of fever and neutropenia in children with cancer and hematopoietic stem-cell transplantation recipients: 2017 update. J Clin Oncol 2017; 35: 2082-2094. https://doi.org/10.1200/JCO.2016.71.7017

15. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005; 6: 2-8. https://doi.org/10.1097/01.PCC.0000149131.72248.E6

16. Ellis M. Febrile neutropenia. Ann N Y Acad Sci 2008; 1138: 329-350. https://doi.org/10.1196/annals.1414.035

17. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001; 345: 1368-1377. https://doi.org/10.1056/NEJMoa010307

18. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care 2010; 14: R15. https://doi.org/10.1186/cc8872

19. Yano K, Liaw PC, Mullington JM, et al. Vascular endothelial growth factor is an important determinant of sepsis morbidity and mortality. J Exp Med 2006; 203: 1447-1458. https://doi.org/10.1084/jem.20060375

20. Taha Y, Raab Y, Larsson A, et al. Vascular endothelial growth factor (VEGF)-a possible mediator of inflammation and mucosal permeability in patients with collagenous colitis. Dig Dis Sci 2004; 49: 109-115. https://doi.org/10.1023/b:ddas.0000011611.92440.f2

21. Karlsson S, Pettilä V, Tenhunen J, et al. Vascular endothelial growth factor in severe sepsis and septic shock. Anesth Analg 2008; 106: 1820-1826. https://doi.org/10.1213/ane.0b013e31816a643f

22. Pickkers P, Sprong T, Eijk LV, Hoeven HVD, Smits P, Deuren MV. Vascular endothelial growth factor is increased during the first 48 hours of human septic shock and correlates with vascular permeability. Shock 2005; 24: 508-512. https://doi.org/10.1097/01.shk.0000190827.36406.6e

23. Hämäläinen S, Juutilainen A, Matinlauri I, et al. Serum vascular endothelial growth factor in adult haematological patients with neutropenic fever: a comparison with C-reactive protein. Eur J Haematol 2009; 83: 251-257. https://doi.org/10.1111/j.1600-0609.2009.01260.x

24. Kalra M, Dinand V, Choudhary S, Sachdeva A, Yadav SP. Serum vascular endothelial growth factor-a levels during induction therapy in children with acute lymphoblastic leukemia. Indian Pediatr 2013; 50: 659-662. https://doi.org/10.1007/s13312-013-0198-6

25. Shapiro NI, Yano K, Okada H, et al. A prospective, observational study of soluble FLT-1 and vascular endothelial growth factor in sepsis. Shock 2008; 29: 452-457. https://doi.org/10.1097/shk.0b013e31815072c1

26. Shapiro NI, Schuetz P, Yano K, et al. The association of endothelial cell signaling, severity of illness, and organ dysfunction in sepsis. Crit Care 2010; 14: R182. https://doi.org/10.1186/cc9290

27. Tsao PN, Chan FT, Wei SC, et al. Soluble vascular endothelial growth factor receptor-1 protects mice in sepsis. Crit Care Med 2007; 35: 1955-1960. https://doi.org/10.1097/01.CCM.0000275273.56547.B8

28. Mankhambo LA, Banda DL; IPD Study Group, et al. The role of angiogenic factors in predicting clinical outcome in severe bacterial infection in Malawian children. Crit Care 2010; 14: R91. https://doi.org/10.1186/cc9025

29. Tsigkos S, Koutsilieris M, Papapetropoulos A. Angiopoietins in angiogenesis and beyond. Expert Opin Investig Drugs 2003; 12: 933-941. https://doi.org/10.1517/13543784.12.6.933

30. Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 2006; 12: 235-239. https://doi.org/10.1038/nm1351

31. Bhandari V, Choo-Wing R, Lee CG, et al. Hyperoxia causes angiopoietin 2-mediated acute lung injury and necrotic cell death. Nat Med 2006; 12: 1286-1293. https://doi.org/10.1038/nm1494

32. Giuliano JS, Lahni PM, Harmon K, et al. Admission angiopoietin levels in children with septic shock. Shock 2007; 28: 650-654. https://doi.org/10.1097/shk.0b013e318123867b

33. Alves BE, Montalvao SAL, Aranha FJP, et al. Imbalances in serum angiopoietin concentrations are early predictors of septic shock development in patients with post chemotherapy febrile neutropenia. BMC Infect Dis 2010; 10: 143. https://doi.org/10.1186/1471-2334-10-143

34. Mimaroğlu E, Çıtak EÇ, Kuyucu N, Eskendari G. The diagnostic and prognostic value of angiopoietins compared with C-reactive protein and procalcitonin in children with febrile neutropenia. Turk J Pediatr 2017; 59: 418-425. https://doi.org/10.24953/turkjped.2017.04.008