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Original Article

Assessment of Angiogenesis in Children with Acute Lymphoblastic Leukemia Based on Serum Vascular Endothelial Growth Factor Assay

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Abstract

Introduction: Vascular endothelial growth factor A (VEGFA) is a key proangiogenic cytokine. The role of angiogenesis in acute lymphoblastic leukemia (ALL) is still unclear. The purpose of the study was to assess angiogenesis in children with ALL based on serum VEGFA level determined at diagnosis and at remission with further participant subdivision into different risk groups. **Materials and Methods:** Forty children, aged 3–12 years (mean age: 8 years) with newly diagnosed ALL, were enrolled in the study. The control group (Group C) was twenty healthy children. According to the risk assessment, they were classified into a standard-risk group, an intermediate-risk group (IRG), or a high-risk group (HRG). **Results:** The median serum VEGFA levels at diagnosis were significantly higher in IRG and HRG as compared to Group C. The VEGFA levels at remission were significantly higher in all study groups, as compared to Group C. The differences in median values of serum VEGFA levels between the study groups both at diagnosis and at remission were not statistically significant. **Conclusions:** The angiogenesis in ALL seems to be intensified at diagnosis as a result of neoplasmatic bone marrow rebuilding and at remission as its intensive recovering.

Keywords: *Acute lymphoblastic leukemia, angiogenesis, children, vascular endothelial growth factor*

Introduction

Angiogenesis is a process essential for growth and development of all human tissues – both healthy and changed morbidly. There are a number of factors which stimulate angiogenesis, and the vascular endothelial growth factor A (VEGFA) has a key role among them. It was proved to be released by endothelial cells, lymphocytes, fibroblasts, macrophages, megakaryocytes, and tumor cells.[1-3] Whereas the importance of neovascularization and angiogenesis for solid tumors and their growth is understandable, the role of these processes in hematopoietic malignancies, especially acute lymphoblastic leukemia (ALL), has still been debated. Some researchers state that proangiogenic cytokines of leukemic origin stimulate angiogenesis in patients with ALL.[4,5] The leukemic cells which have VEGFA surface receptors may be susceptible to both autocrine and paracrine stimulation.[5] This promotes both the blood vessel formation within the bone marrow and the proliferation of malignant cells. On the other hand, there are studies showing that leukemic cell clones inhibit angiogenesis and destroy normal progenitor bone marrow cells responsible mainly for VEGFA production.[6,7] The described discrepant opinions justify the need of further research on angiogenesis in ALL.

The purpose of the study was to assess angiogenesis in children with ALL based on serum VEGFA level determined at diagnosis and at remission (day 33 of treatment), with further participant subdivision into different risk groups.

Materials and Methods

Procedure

Forty children (23 boys – 57.5%; 17 girls – 42.5%) aged 3–12 years (mean age: 8 years), with newly diagnosed ALL, were enrolled in the study. All participants were treated according to the ALL Intercontinental 2002 (ALL IC 2002) protocol. They were classified according to the risk into one of the three groups: a standard-risk group (SRG; n = 18), an intermediate-risk group (IRG; n = 12), and a high-risk group (HRG; n = 10). The risk assessment included patient age at diagnosis, white blood count at diagnosis,

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blast cell count per 1 μL of peripheral blood on day 8 of treatment, blast cell count (%) in bone marrow on day 15 and 33 of treatment, minimal residual disease on day 15 as well as cytogenetic and biomolecular investigation findings [Table 1].

The exclusion criteria were children with known additional physiological and pathological processes such as menstruation, previous injuries, or surgery, which could further enhance angiogenesis.

The control group (Group C) consisted of 20 healthy children and patients from pediatric clinic with functional gastrointestinal tract disturbances (13 boys – 65%, 7 girls – 35%) aged 2–15 years (mean age: 8 years).

The serum level of VEGFA was determined at diagnosis and at remission.

Fasting blood samples for laboratory analyses were collected from the ulnar vein using a G0.8 needle, after prior skin disinfection with 70% alcohol. To assess the serum VEGF concentration, additional 2 cm^3 blood samples were collected from each participant/control during a routine phlebotomy. Clotted samples were centrifuged for 20 min at 2000 rpm. The obtained serum samples were frozen at −80°C. The analyses were performed using the Human VEGFA Quantikine Colorimetric Sandwich ELISA kit (R&D Systems).

The serum VEGFA levels in study participants were compared with those of the controls.

All participants and controls as well as their parents/carers were provided detailed and sufficient information on the method and purpose of the study. A written informed consent was obtained from parents for collecting an additional 2 cm^3 blood sample for observational research purposes.

Statistical analysis

Since the distribution of variables was considerably different from the normal distribution (Kolmogorov–Smirnov test), they were described using the median (range); the hypotheses were verified using the nonparametric tests. The differences between the two groups in relation to individual numerical variables were verified using the Mann–Whitney U-test. $P < 0.05$ for the determined differences and associations was considered statistically significant.

Results

The median of serum VEGFA at diagnosis was comparable between the SRG and the Group C and was significantly higher in IRG and HRG as compared to the Group C. At remission, the median of serum VEGFA level was significantly higher in all study groups, as compared to the Group C [Table 2].

Table 1: The clinical characteristic examinated groups

	SRG (*n*=18)	IRG (*n*=12)	HRG (*n*=10)
Age years			
<1	-	-	-
≥1-<5	18	-	2
≥5	-	12	8
Gender			
Boys	12	7	4
Girls	6	5	6
WBC (at diagnosis)			
<20.0 ×109/L	18	-	4
≥20.0 ×109/L	-	12	6
Peripheral blasts 8 days			
<1000.0/uL	18	12	-
≥1000.0/uL	-	-	3
Bone marrow 15 days			
M1	18	3	-
M2	-	9	6
M3	-	-	4
MRD bone marrow 15 days			
<0.1%	18	5	1
≥0.1%-<10%	-	7	5
≥10.0%	-	-	4
Bone marrow 33 days			
M1	18	12	10
M2	-	-	-
M3	-	-	-
t (9.22)	-	-	3
t (4.11)	-	-	-
Hypodiploidy	-	-	-
Peripheral lymphadenopathy	14	11	8
Hepatosplenomegaly	15	12	10

Bone marrow M1 - ≤5% blasts; Bone marrow M2 - 5% – 25% blasts; Bone marrow M3 - ≥25% blasts. SRG - Standard-risk group; IRG - Intermediate-risk group; HRG - High-risk group; WBC - White blood count; MRD - Minimal residual disease

The median of serum VEGFA levels in SRG was significantly higher at remission, as compared to diagnosis. The median values of serum VEGFA at remission were also higher compared to diagnosis in IRG and HRG, but the differences were not statistically significant [Table 3].

The differences in median values of serum VEGFA levels between the groups both at diagnosis and at remission were not statistically significant [Table 3].

Discussion

The role of angiogenesis in cancer has been studied for many years. It can be assessed by means of different markers, for example, serum, plasma, or urine concentration of proangiogenic cytokines, the expression of proangiogenic cytokines and their receptors (both surface and soluble ones) determined using the polymerase

chain reaction (PCR), as well as the microvascular density (MVD) count which reflects changes in blood vessel formation.[8-11]

Progenitor cells, endothelium, and stroma of normal bone marrow were shown to express proangiogenic cytokines.[7,10,12] The said cytokines affect stem cells and entire bone marrow microenvironment by both autocrine and paracrine stimulation.[13,14] The megakaryocyte-rich areas of human bone marrow present with particularly high MVD and VEGF mRNA level.[10]

Based on the above data, a hypothesis was postulated that the level of the key angiogenesis triggering cytokine, i.e., VEGFA, was low in patients with ALL due to the destruction of both bone marrow stem cells and bone marrow microenvironment by the clones of leukemic cells.[6] Others, however, hypothesized that the existence of soluble VEGF receptor in human serum and the presence of VEGF receptor on leukemic cells may induce their interaction, thus decreasing serum VEGF level.[7,15]

Table 2: The median values of serum vascular endothelial growth factor levels in the examinated groups and control group

	VEGFA (pg/ml)	Diagnosis versus Group C (*P*)	33 days versus Group C (*P*)
Group SRG (*n*=18)			
Diagnosis	121.4 (23.6-456.3)	NS	<0.05
33 days	296.0 (31.7-796.8)		
Group IRG (*n*=12)			
Diagnosis	245.6 (22.3-684.4)	<0.05	<0.05
33 days	424.1 (32.2-905.6)		
Group HRG (*n*=10)			
Diagnosis	228.5 (81.3-389.8)	<0.05	<0.05
33 days	318.8 (82.0-796.7)		
Group C (*n*=20)	144.6 (32.7-237.9)		

VEGFA – Vascular endothelial growth factor A; SRG – Standard-risk group; IRG – Intermediate-risk group; HRG – High-risk group; NS – Not significant

This hypothesis was confirmed by Kalra *et al*.,[7] who showed significantly lower VEGF levels at diagnosis as compared to remission in thirty children with ALL. In addition, according to them, low VEGF levels in pediatric nonresponders to treatment may, for instance, reflect the high expression of VEGF receptor on leukemic cells. As a result, more VEGF is bound to the receptors and its serum level decreases.[7] Similarly, Yetgin *et al*.[16] and Aref *et al*.[17] confirmed significantly lower VEGF levels at diagnosis as compared to remission in their studies. Our research appears to reproduce their findings. We showed lower serum VEGFA median at diagnosis as compared to remission. The observed difference was statistically significant only in the SRG. In two other groups – IRG and HRG, serum VEGFA levels were also lower at diagnosis as compared to remission, but the differences were not statistically significant.

According to the research carried out by the Children's Oncology Group,[18] the longest event-free survival was observed in those children with ALL, who presented with lower VEGFA levels both at diagnosis and at the remission. In our research, the SRG participants with most favorable prognosis actually presented with the lowest VEGFA levels at diagnosis and at remission, as compared to other groups with poorer prognosis (IRG, HRG). At remission, median VEGFA in SRG was significantly higher as compared to diagnosis, which may be consistent with intensive bone marrow rebuilding and VEGFA production by the progenitor cells without its simultaneous uptake by malignant cell receptors.

However, there are studies reporting contradictory findings. Chand *et al*.[19] who studied angiogenesis in a group of 15 children with ALL showed significantly higher serum VEGF level and MVD determined at diagnosis, as compared to the same values at remission. According to the report by Perez-Atayde *et al*.[20] published in 1997, the bone marrow of children with ALL presented with increased microvascular density (MVD). Hussong *et al*.[21] reported similar findings evaluating that bone and marrow specimens

Table 3: The median values of serum vascular endothelial growth factor levels in the groups both at diagnosis and at remission

	VEGFA (pg/ml)	Diagnosis versus remission (*P*)	Diagnosis, group comparison (*P*)	Remission, group comparison (*P*)
Group SRG (*n*=18)				
Diagnosis	121.4 (23.6-456.3)	<0.05	Group SRG versus	Group SRG versus
33 days	296.0 (31.7-796.8)		group IRG NS	group IRG NS
Group IRG (*n*=12)				
Diagnosis	245.6 (22.3-684.4)	NS	Group SRG versus	Group SRG versus
33 days	424.1 (32.2-905.6)		group HRG NS	group HRG NS
Group HRG (*n*=10)				
Diagnosis	228.5 (81.3-389.8)	NS	Group IRG versus	group IRG versus
33 days	318.8 (82.0-796.7)		group HRG NS	group HRG NS

VEGFA – Vascular endothelial growth factor A; SRG – Standard-risk group; IRG – Intermediate-risk group; HRG – High-risk group; NS – Not significant

obtained from children with leukemia. According to Chand *et al*.,[21] patients with different hematologic malignancies, including ALL, who present with elevated MVD and VEGF at baseline, may make good candidates for antiangiogenic therapy, especially if they are poor responders to the first-line treatment.

There are studies which showed a lack of differences between angiogenesis biomarker levels in patients with ALL at different stages of treatment. Leblebisatan *et al*.[8] used PCR to assess VEGF expression in leukemic cells of 28 children. They did not observe significant differences between the assays at diagnosis and recurrence at remission.

Our research points to the increased angiogenesis in ALL both at diagnosis and at remission, with its completely different triggers implicated at different time points. Significantly lower VEGFA values in healthy children as compared to the IRG and HRGs suggest the role of leukemic cells in VEGFA production. Higher VEGFA levels at remission in all children with ALL as compared to healthy controls may be indicative of intensive bone marrow regeneration and VEGFA production by normal bone marrow progenitor cells.

Our findings as well as other studies of this case sufficiently prove that angiogenesis in children with ALL has not been fully explained yet, so further research in large, uniform pediatric population is legitimate and essential. According to some researchers, serum levels of proangiogenic cytokines in patients newly diagnosed with ALL may be seen as a source of knowledge on its etiopathogenesis, which could later help monitor treatment response or affect treatment choices.[8,13,14]

Conclusions

The significantly higher level of serum VEGF in children with ALL in comparison with healthy control, both at the diagnosis and in the remission, could suggest the intensification of angiogenesis in the bone marrow, at diagnosis due to neoplasmatic proliferation, at remission due to physiological, intensive bone marrow rebuilding.

There is a tendency for higher serum VEGF level in the groups with less satisfying prognosis, which suggests further examinations on wider groups of patients.

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Conflicts of interest

There are no conflicts of interest.

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