Journal of the Egyptian Society of Haematology & Research, Vol. 15, No. 1, March: 17-21, 2019

Increased Angiogenesis and Response to Induction Therapy in De Novo Egyptian Pediatric Acute Lymphoblastic Leukemia Patients

MARIAM M. ELHADDAD, M.D.[1]; MAHMOUD F. GUIBALY, M.D.[1]; BASMA M. ELGAMAL, M.D.[1]; ALAA M. ELHADDAD, M.D.[2]; ABDELHAMID M. FOUAD, M.D.[3]; NORA A. GOUDA, M.D.[4] and NAGLAA M. HASSAN, M.D.[1]

The Department of Clinical Pathology[1], Pediatric Oncology Department[2], Medical Oncology Department[3] and Medical Statistics Department[4], National Cancer Institute, Cairo University, Egypt

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is malignant transformation and proliferation of lymphoid progenitor cells in bone marrow, blood and extramedullary sites. It is the most common leukemia in children.

Angiogenesis is the formation of new blood vessels from preexisting vessels and is a normal and highly regulated physiological process throughout the body. Tumors have traditionally been the most extensively studied angiogenic-dependent diseases.

Purpose: This study was designed to investigate impact of increased angiogenesis measured by microvascular density (MVD) using anti-CD34 marker on the response to induction therapy in newly diagnosed Egyptian pediatric acute lymphoblastic leukemia patients.

Methods: Forty de novo pediatric ALL patients coming to National Cancer Institute (NCI), Cairo University from May 2017 to June 2019, were included. Bone marrow aspirations (BMA) and bone marrow biopsy (BMB) specimens were obtained from all patients at diagnosis: BMA smears were stained with Leishman stain and cytochemical stains and BMB were processed then stained with haematoxylin and eosin, reticulin and CD34 immunohistochemical (IHC) stain for assessment of MVD.

All patients received total 15 induction therapy and assessed at end of phase 2 induction.

Results: Angiogenesis ranged from 1 to 20 vessels per high power field (HPF), with median of 6 vessels per high power field. From our experience in NCI, Cairo University, cases with count above 6 vessels/HPF are considered having increased angiogenesis.

Out of the 40 patients included in the study, 19 patients (47.5%) were having increased angiogenesis (>6 vessels/HPF) while 21 patients (52.5%) were having normal vessel count (≤6 vessels/HPF).

Conclusions: In this study we found that increased angiogenesis had no impact on response to induction therapy with Total XV protocol in childhood ALL. Still large prospective trials are needed to confirm or deny this conclusion.

Key Words: *Angiogenesis – Childhood ALL – Induction therapy – Response – Total XV protocol.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant clonal disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow, blood and extramedullary sites **[1]**. It is the most common type of cancer and leukemia in children accounting for up to 80% of leukemias in this age group and 20% of leukemias in adults **[2]**.

Angiogenesis is the process through which novel blood vessels are formed from pre-existing ones and it is involved in both physiological and pathological processes of the body. Tumor angiogenesis is a crucial factor associated with tumor growth, progression, and metastasis. That is why there were great interestsfor the development of anti-angiogenic strategies that could inhibit tumor vascularization. Approaches comprise either the administration of anti-angiogenic drugs that target and block the activity of proangiogenic factors or combining anti-angiogenic agents with chemotherapy or immunotherapy **[3]**.

PATIENTS AND METHODS

This study included 40 de novo Egyptian pediatric ALL patients attending National Can-

cer Institute (NCI), Cairo University; during the time period from May 2017 to June 2019.

The study was conducted after institutional ethical clearance. Written informed consent was obtained from all patients and/or their parents.

Patients included aged between 2 to 18 years old, with confirmed diagnosis of ALL and were classified according to morphology, cytochemistry, immunophenotyping and cytogenetics.

Patients were monitored regularly in the oncology outpatient clinics and treated with the current chemotherapy protocols (Total XV induction therapy).

Patients were classified to low, intermediate or high risk based on age, White blood count (WBC) count, immunophenotype, and central nervous system (CNS) involvement at diagnosis, in addition their cytogenetic and molecular status **[4]**.

Bone marrow aspiration (BMA) and Bone marrow biopsy (BMB) were obtained from all patients at diagnosis. The BMA smears were stained with Leishman stain and cytochemical stains, namely, myeloperoxidase (MPO) and Sudan black (SBB).

BMB were formalin fixed and paraffin embedded and cut into 3-4μm thick sections then stained with haematoxylin and eosin staining, as well as reticulin and CD34 immunohistochemical (IHC) stain.

Microvessel Density (MVD) Calculation:

Estimation of MVD was accomplished through the following steps done for BM sections as follows:

Using a research binocular light microscope [Leica, DM – 750], the tested immuno-stained slides were initially scanned at 100 x magnification to identify the section area of the slide, check the staining quality, verify the distribution pattern of the highlighted microvessels and to locate regions of higher vascular concentrations (i.e. hot spots) **[5]**.

Ten hotspots were chosen and numbers of microvessels were counted in each of these hotspots at 400x magnification. To ensure the accuracy of the method, each stained sample was reviewed by 2 separate hematopathologist, in a blinded fashion. Morphologic analysis was performed carefully to ensure vessel specificity of the CD 34-stained stroma considered for analysis. The MVD was expressed as the mean number of microvessels per field **[6]**.

Quantification of microvessels was performed according to the following rules [5]:

1- Any IHC-stained (CD34 +ve) individually scattered endothelial cell was considered as a single distinct countable microvessel.

2- Any IHC-stained endothelial cell cluster (whether arranged in a complete or incomplete vascular structure, with or without a lumen and clearly separated from adjacent microvessels, blasts, and other marrow elements) was considered also as a single distinct countable microvessel.

3- The presence of a lumen (with or without RBCs) was not necessary for microvessels morphological identification but considered only as a helpful feature.

4- All immuno-stained highlighted microvessels (whether crowded within the “Hot spots” or randomly individually dispersed among the intertrabecular hemopoietic areas) were included into the count. Those encountered within the BM trabeculae were not included into the count.

5- All immuno-stained microvessels (whether located among the malignant hemopoietic infiltrates or among the non-infiltrated normal hemopoietic areas) were included into the count.

Response assessment:

Complete remission (CR) is defined as <5% marrow blasts with peripheral blood (PB) count recovery, evidence of normal hematopoiesis, and absence of extramedullary disease.

Statistical methods:

Data were analyzed using SPSS with statistical package version 17. Qualitative variables will be presented as proportions and quantitative variables will be presented as mean ± standard deviation (SD) or median and range as appropriate. The comparison between qualitative variables will be done using Chi-square test or Fisher's exact as appropriate and *p* less than 0.05 will be considered. Qualitative data were expressed as frequency and percentage. Survival analysis was done using Kaplan-Meier method.

RESULTS

I- *Patients characteristics:*

Clinical and hematological characteristics of the 40 patients are listed in Table (1).

Risk stratification of the 40 patients included in the study:

In this study, 25 patients (62.5%) were standard risk, 12 patients (30%) showed low risk and 3 patients (7.5%) were high risk.

Treatment protocol:

All the 40 patients started their induction of remission therapy, 38 patients (95%) were given induction total XV therapy protocol, and the remaining 2 t(9; 22) (BCR-ABL fusion gene) positive patients were given an additional targeted therapy (Glivec), (total XV+Glivec).

Assessment of response:

Thirty one out of 40 patients (77.5%) were in CR at day 15 (early assessment) of induction of therapy, 28 patients (70%) continued induction till day 42 (late assessment) and were in CR. Four out of 28 patients who had been in CR1 (14.3%) had relapsed: 1 out of 4 patients had an isolated CNS relapse and received re-induction therapy with German protocol, the 3 other patients had bone marrow (BM) relapse and were given re-induction with FLAGM protocol. Three out of 4 relapsed patients were in second CR, and 1 patient had died.

II- *Relation between angiogenesis and clinical parameters in our 40 patients:*

Statistical relation between angiogenesis and clinical parameters of patients included in the study at diagnosis is shown in Table (2).

III- *Relation of angiogenesis to the response to induction therapy:*

Among our 40 patients 31 patients achieved CR1 at day 42 of induction therapy of whom 17 patients were having increased MVD, and 3 patients did not achieve CR1, all the 3 were having normal MVD. Three patients (1 patient with normal MVD and 2 were having increased MVD) achieved CR2 after changing therapy protocol and 4 patients (2 patients were having increased MVD and the other 2 were having normal MVD) relapsed after induction therapy; still no statistically significant relationship was found neither between response to induction therapy nor relapse to angiogenesis as shown in Table (3).

Table (1): Clinical and hematological findings at diagnosis.

	N=40	%
Age:		
Median (range) (years)	8.5 (2.0-18.0)	
Sex:		
Male	31.00	77.50
Female	9.00	22.50
Initial CBC:		
TLC:		
Median (range) ($x10^9$/L)	9850 (1400-109000)	
HB:		
Median (range) (mg/dl)	8.6 (4.1-16.0)	
PLT:		
Median (range) ($x10^9$/L)	53000 (4000-279000)	
PB Blasts:		
Median (range) (%)	25.00% (0.0-88.0)	
Initial BMA:		
BM blasts:		
Median (range) (%)	88.00% (17.00-99.0)	
BM Cellularity:		
Normocellular	9.00	22.50
Hypocellular	8.00	20.00
Hypercellular	23.00	57.50
TP53:		
Both copies of TP53	35.00	87.50
3 copies of TP53	3.00	7.50
del of TP53	2.00	5.00
IPT:		
Pre B-ALL	32.00	80.00
c ALL	8.00	20.00

Table (2): Relation between angiogenesis and clinical parameters of study patients.

	Angiogenesis ≤6		Angiogenesis >6		*p*-value
	N	%	N	%	
Age group (yrs):					
2-10	11	52.4	10	47.6	0.987
>10	10	52.6	9	47.4	
Sex:					
Male	15	71.4	16	84.2	0.457
Female	6	28.6	3	15.8	
Organomegaly:					
No	8	44.4	10	55.6	0.356
Yes	13	59.1	9	40.9	
CSF:					
No	19	50.0	19	50.0	0.488
Yes	2	100.0	0	0.0	
LNs:					
No	11	55.0	9	45.0	1.000
Yes	10	50.0	10	50.0	

Table (3): Relation between angiogenesis and response to induction therapy.

	Angiogenesis ≤6		Angiogenesis >6		*p*-value
	N	%	N	%	
CR1:					
No	3	17.6	0	0.0	0.227
Yes	14	82.4	17	100.0	
CR2:					
No	0	0.0	0	0.0	0.341
Yes	1	100.0	2	100.0	
Relapse:					
No	7	77.8	14	87.5	0.60
Yes	2	22.2	2	12.5	
Status:					
Alive	7	33.3	14	73.7	0.011
Dead	14	66.7	5	26.3	
Death:					
Early	10	71.4	3	60.0	1.00
Late	4	28.6	2	40.0	

DISCUSSION

In this study, detection of BM microvessels was performed by IHC staining using the mouse antihuman CD34 which highlights the endothelial cells. Babarovic et al., 2012 **[5]** noticed that the best IHC results were obtained with anti-CD34 monoclonal antibody as CD31 and factor VIII are expressed in a big population of bone marrow cells, including megakaryocytes and myeloid cells.

In the current study, we found no association between angiogenesis (MVD) and both age and sex of the patients, this goes well with other statistical analysis reported by Pule et al., 2002 **[7]** and Noren–Nystrom et al., 2009 **[8]** and who also did not reveal any significant association between MVD and age or sex of the patients in ALL.

This work did not also reveal any significant difference in angiogenesis (MVD) in relation to the presence of organomegaly and lymphadenopathy. To the best of our knowledge, no previous studies addressed the relation of these parameters with MVD in acute leukaemia.

The increase in cellularity and MVD were noticed in our ALL patients, since the majority of cellularity is composed of blasts, a correlation between MVD and cellularity may be expected. However, the increased angiogenesis was not related to neither BM cellularity nor to BM blast cell percentage in ALL patients included in our study. These results may suggest that the increase in BM vascularity is not related to BM degree of cellularity; to our knowledge no published studies were available discussing this relation in ALL. But, this goes well with Kuzu et al., 2004 **[9]** who found no statistical relation between MVD and percentage of BM blast cells in AML.

This study shows no relation between increased angiogenesis and WBC count in ALL, this is consistent with Noren-Nystrom et al., 2008 **[8]** who also found no correlation between microvascular density MVD and WBC count in ALL.

In newly diagnosed ALL patients we found that there is no relation between increased BM angiogenesis (MVD) and response to induction therapy nor relapse or patient survival, this was in accordance with Pule et al., 2002 **[7]** who also demonstrated no association between high MVD and prediction of relapse. In contrast to a study done by Todorovic et al., 2011 **[10]** found that the initial values of MVD had a positive correlation with OS and leukaemia free survival.

Kuzu et al., 2004 **[9]** and Shih et al., 2009 **[11]** found a significant increase in MVD in the BM of AML patients, this is particularly significant when considering the strong positive correlation between increased BM vasculature and OS in AML. A high MVD predicted for poor prognosis and suggests that blood vessel-AML interactions may contribute to refractory disease.

In most patients with myelofibrosis with myeloid metaplasia, Mesa et al., 2000 **[12]** demonstrated an increase MVD compared with patients with either polycythemia vera or essential thrombocythemia, they also detected that increased angiogenesis, along with other factors, was a highly significant risk factor for survival.

In patients with multiple myeloma (MM), increased BM angiogenesis and prognosis are well related, and MVD is considered as an independent prognostic factor for OS together with beta2-microglobulin and C-reactive protein **[13,14]**.

Conclusion:

We found that there was no relation between increased angiogenesis expressed by MVD and

response to induction therapy in newly diagnosed Egyptian pediatric ALL patients. However, further large prospective studies are recommended to prove or deny this finding.

REFERENCES

1- Seiter K. Acute Lymphoblastic Leukemia (ALL); Medscape oncology; Updated: Oct. 2020; 26.

2- Zuckerman T. Rowe J. Pathogenesis and prognostication in acute lymphoblastic leukemia. F1000 Prime Rep. 2014; 6: 59. doi: 10.12703/P6-59.

3- Teleanu R, Chircov C, Grumezescu A, Teleanu D. Tumor Angiogenesis and Anti-Angiogenic Strategies for Cancer Treatment; J. Clin. Med. 2020; 9, 84; doi: 10.3390/jcm9010084.

4- Cooper S, Brown P. Treatment of pediatric acute lymphoblastic leukemia. Pediatr Clin North Am. 2015; 62 (1): 61-73.

5- Babarovic E, Valkovic T, Stifter S, et al. Assessment of bone marrow fibrosis and angiogenesis in monitoring patients with Multiple Myeloma, Am J Clin Pathol. 2012; 137: 870-878.

6- Chavez-MacGregor M, Aviles-Salas A, Green D, et al. Angiogenesis in the Bone Marrow of Patients with Breast Cancer. Clin Cancer Res. 2005; 11 (15).

7- Pule M, Gullmann C, Dennis D, et al. Increased angiogenesis in bone marrow of children with acute lymphoblastic leukaemia has no prognostic significance. British Journal of Haematology. 2002; 118: 991-998.

8- Noren-Nystrom U, Heyman M, Frisk P, et al. Vascular density in childhood acute lymphoblastic leukaemia correlates to biological factors and outcome. Br J Haematol. 2009; 146: 521-530.

9- Kuzu I, Beksac M, Arat M. Bone marrow microvessel density (MVD) inadult acute myeloid leukaemia (AML): Therapy induced changes and effects on survival. Leukaemia Lymphoma. 2004; 45: 1185-90.

10- Todorovic M, Balint B, Radisavljevic Z, et al. Microvessel density evaluation inacute ymphoblastic leukemia – a study of angiogenesis. Bilt Transfuziol. 2011; 57 (1-2): 49-51.

11- Shih T, Hou H, Liu C, et al. Bone marrow angiogenesis magnetic resonance imaging in patients with acute myeloid leukemia: Peak enhancement ratio is an independent predictor for overall survival. Blood. 2009; Vol. 113, No. 14, pp. 3161-3167.

12- Mesa RA, Hanson CA, Rajkumar SV. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. Blood. 2000; 96: 3374-3380.

13- Rajkumar SV, Mesa RA, Fonseca R, et al. Bone marrow angiogenesis in 400 patients with monoclonal gammopathy of undetermined significance, multiple myeloma, and primary amyloidosis. Clin Cancer Res. 2002; 8: 2210-2216.

14- Alexandrakis MG, Passam FH, Dambaki C, et al. The relation between bone marrow angiogenesis and the proliferation index Ki-67 in multiple myeloma. J Clin Pathol. 2004; 57: 856-860.