Immunohistochemical study of bone marrow angiogenesis using CD34 in adult acute lymphoblastic leukemia and its correlation with various pathological, laboratory and clinical parameters

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Summary:

Background: In recent years, bone marrow angiogenesis is indicated to be involved in the pathogenesis and progression of certain hematological malignancies like acute leukemia, lymphomas, and multiple myeloma. Recent studies have suggested that bone marrow angiogenesis plays an important role in the pathogenesis of adult acute lymphoblastic leukemia and also has prognostic value in the disease.

Objectives: at the present study, bone marrow angiogenesis in ALL will be examined using immunohistochemical staining for CD34, and this will be correlated with various pathological, laboratory and clinical parameters.

Patients and methods: A retrospective cross-sectional study was done on 60 patients with acute lymphoblastic leukemia (32 males & 28 females) compared with 20 controls (anemic patients), all recruited at the Medical City Hospital/ Teaching Laboratories/ Baghdad from January 2010 to December 2012. The bone marrow biopsy of each was re-examined histologically. BM angiogenesis was studied by immunohistochemical staining for CD34 to identify microvessels.

Results: The bone marrow microvessel density was markedly and significantly increased in patients with acute lymphoblastic leukemia compared with bone marrow controls ($p = 0.0001$). There was a significant association between angiogenesis grade III and WBC count more than 30×109/L ($P > 0.0001$).

Conclusions: Angiogenesis in ALL was significantly higher than control group. Increase angiogenesis confirmed by IHC was significantly correlated with high WBC at diagnosis. These observations suggest that the combination of anti antiangiogenic therapy might apply to leukemia.

Key words: ALL; angiogenesis; CD34

Introduction:

Angiogenesis is the formation of new vessels from an existing network of vasculature, and it plays a significant role in a variety of physiologic and pathophysiologic processes like tumor growth and spread.1 Angiogenesis is recognized as a crucial component in the growth and metastatic spread of solid tumors, and it is a complicated development in the disease process that involves the degradation of extracellular matrix proteins and the activation, proliferation, and migration of endothelial cells and pericytes in a multistep manner.2

Recently, it has also been recognized that dysregulation of angiogenesis constitutes an important step in the development and progression of hematologic malignancies and that leukemias may invade and proliferate in the bone marrow and other organs by mechanisms similar to those reported in solid tumors.3 Measurement of microvessel density by immunohistochemistry like CD34, has been widely used for assessing the extent of angiogenic activity.4

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Patients, materials and methods

A retrospective cross-sectional study; where by archival paraffin-embedded tissue blocks along with the clinical and hematological records of sixty patients with acute lymphoblastic leukemia were obtained from the Department of Hematology of the Medical City Teaching Laboratories in the period from January 2010 to December 2012. The bone marrow biopsies were performed at diagnosis. The patients were newly diagnosed and did not receive prior treatment. All relevant clinical including lymphadenopathy, splenomegaly and hepatomegaly, and laboratory data available for all patients were reviewed. Paraffin-embedded tissue blocks of twenty control individuals along with their hematological reports were also collected. All the control bone marrows were negative for infiltrative lesions and were obtained from patients with anemia due to iron or vitamin B12 deficiencies and thrombocytopenia. All cases (patients and control) were subjected to

immunohistochemical study of CD34 in the Central Public Health Lab/Baghdad.

Microvessel Staining: Bone marrow biopsy specimens used in this study were prepared from paraffin-embedded blocks. Immunohistochemical staining for CD34 was performed by a labeled streptavidin-biotin peroxidase method on the automated immunohistochemistry stainer (Danemark) using buffers and detection reagents supplied by the manufacturer. The primary antibody CD34 class 2 (DAKO Denmark) monoclonal mouse (QBEnd 10) IgG1 Kappa; diluted against 0.05 mol/L Tris/Hcl was incubated with tissue sections for 20 min. The LSAB + detection kit (DAKO) was used for antigen visualization; sections were counter stained with a light hematoxylin and then coverslipped. Paraffin sections of Hemangioma were run with each batch to serve as a positive control, and a section stained with nonimmune rabbit immunoglobulin was used as a negative control for each sample tested.

Estimation of microvessel density and its grading: Slides were first scanned at 100× magnification, and five areas of maximum microvessels density (MVD) called hot spots were identified at 200× magnification on each slide. In each of these hot spots, microvessels (capillaries and small venules) were counted at 400×. In each case, means of the hot spots were counted. Microvessels were identified as endothelial cells either singly or clustered in nests or tubes, clearly separated from one another, with or without lumen not exceeding 10 mm (i.e., not more than 1.5 times the endothelial cell nucleus) in transverse diameter. Areas occupied by larger vessels and vessels in the periosteum were excluded.5

Each slide (cases as well as controls) were assigned an angiogenesis grade.

Grading of test cases was performed taking into account the MVD of controls, such that the MVD of all the controls included was Grade I MVD of the test cases. MVD was graded as follows 5:

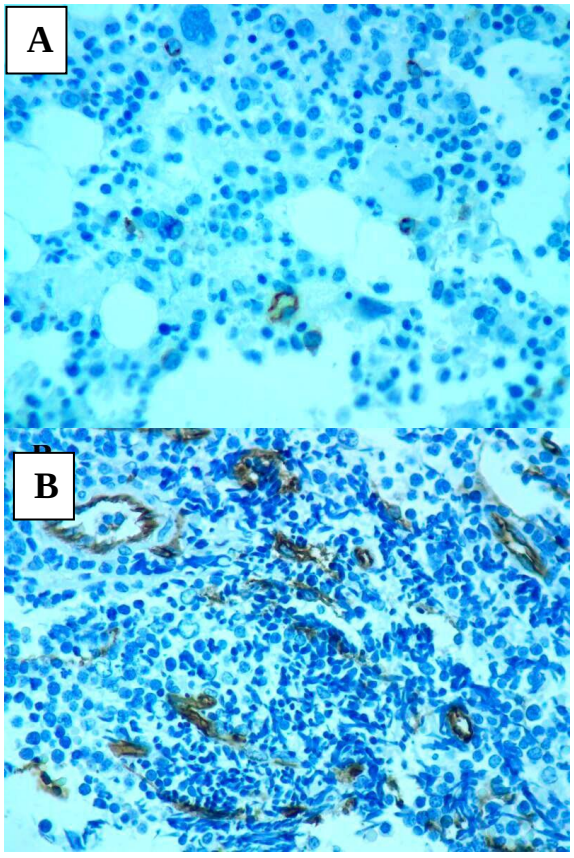
1. Grade I: ≤ 25 microvessels. 2. Grade II: > 25 microvessels but ≤ 50 microvessels. 3. Grade III: > 50 microvessels/high power field (×40).5

Statistical analysis: Statistical analysis was performed with the SPSS 20 statistical software program. Univariate data were summarized using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. Associations between categorical variables were assessed via crosstabulation and chi-square. Exact tests were used to calculate the p value. In all statistical analyses, a p value < 0.05 was considered to be significant.

Results:

A total of 60 patients with ALL; 32 males and 28 females were included in this study. Ages ranged between 15 and 55 years with a mean age of 24.25 ±9.4 years and the male: female ratio was1.1:1.

The bone marrow microvessel density was markedly and significantly increased in patients with acute lymphoblastic leukemia compared with bone marrow controls (p=0.0001) (Figure 1A & B) and table 1.



(Figure 1): Bone marrow section (×400) showed few microvessels in control group (A) and numerous microvessels in ALL patients (B) (×400) [IHC, CD 34].

Table 1: Differences in MVD between patients and control.

Subject	N	Range MVD	Mean ± (SD) MVD	P=0.0001
Patients	60	17-63	33.95 ± (13.7)	
Control	20	4-13	8.30 ± (3.3)	

The distribution of the different percentages of angiogenesis grades among ALL patients is shown in table 2.

Table 2: Angiogensis grade of ALL Patients.

	Number of patients/Frequency
Grade-I: (Less than 25 MV/ HPF)	19 (31.7%)
Grade-II: (25-50 MV/ HPF)	28 (46.7%)
Grade-III: (More than 50 MV/ HPF)	13 (21.7%)
Total	60 (100%)

There was no significant association between angiogenesis grade and splenomegaly (table 3), lymphadenopathy (table 4) and hepatomegaly (table 5).

Table 3: Correlation between splenomegaly and angiogenesis grades.

		Angiogenesis grade			Total	P = N.S
		Grade-I	Grade-II	Grade-III		
Splenomegaly	Present	18 (36%)	21 (42%)	11 (22%)	50 (100%)	
	Absent	1 (10%)	7 (70%)	2 (20%)	10 (100%)	
Total		19 (31.7%)	28 (46.7%)	13 (21.7%)	60 (100%)	

Table 4: Correlation between lymphadenopathy and angiogenesis grades.

		Angiogenesis grade				P = N.S
		Grade-I	Grade-II	Grade-III	Total	
Lymphadenopathy	Present	15 (34.1%)	19 (43.2%)	10 (22.7%)	44 (100%)	
	Absent	4 (25%)	9 (56%)	3 (18.8%)	16 (100%)	
Total		19 (31.7%)	28 (46.7%)	13 (21.7%)	60 (100%)	

Table 5: Correlation between hepatomegaly and angiogenesis grades.

		Angiogenesis grade				P = N.S
		Grade-I	Grade-II	Grade-III	Total	
Hepatomegaly	Present	12 (32.4%)	17 (45.9%)	8 (21.6%)	37 (100%)	
	Absent	7 (30.4%)	11 (47.8%)	5 (21.7%)	23 (100%)	
Total		19 (31.7%)	28 (46.7%)	13 (21.7%)	60 (100%)	

There was a significant association between angiogenesis grade III and WBC count more than 30×109/L ($P < 0.0001$) (table 6).

Table 6: Correlation between WBC and angiogenesis grades. P = 0.0001

		Angiogenesis grade			
		Grade-I	Grade-II	Grade-III	Total
WBC	Less than 30 (×109/L)	19 (41.3%)	27 (58.7%)	0 (0.0%)	46 (100%)
	More than 30 (×109/L)	0 (0.0%)	1 (7.1%)	13 (92.9%)	14 (100%)
Total		19 (31.7%)	28 (46.7%)	13 (21.7%)	60 (100 %)

Discussion:

Dysregulation of angiogenesis was found to have a major impact on leukemia growth and to constitute an important step in the development and progression of hematological malignancies, including leukemia. Increasing evidence indicated that agiogenesis markers play a role in the pathogenesis of patients with ALL6, 7, 8, 9, 3.

This study confirmed a significant increase of bone marrow angiogenesis in ALL compared with control subjects (table 1). This was similar to that reported by other workers who found high microvessels by IHC6, 7, 8, 10, 11.

A number of studies have demonstrated an important prognostic role of angiogenesis in ALL8, 9. One study of adult ALL showed that patients who express high levels of vascular endothelial growth factor (VEGF), which is a proangiogenic factor, have poor survival 10. Another study showed that high expression of VEGF by leukemic cells was found to be significantly associated with poor prognosis 9.

This study found no correlation between lymphadenopathy, splenomegaly and hepatomegaly and angiogenesis grade. Other workers confirm similar result 9. One Iraqi study showed that there was a significant relation between splenomegaly and lymphadenopathy in adults ALL patients and poor prognosis 12.

There was a significant association between angiogenesis grade III and WBC count more than 30×109/L ($P > 0.0001$). An elevated WBC count at diagnosis (above 30-50×109/L) has been confirmed in various trials as a poor prognostic feature14, 15.This indicate that there was a significant correlation between one of the poor prognostic features of ALL and advanced grade of angiogenesis 16, 3.

In conclusion; angiogenesis in ALL was significantly higher than control group and it may have an unfavorable prognosis in ALL.

References

1. *Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971; 285: 1182-1186. (IVSL)*

2. *Birgit Mosch, Bettina Reissenweber, Christin Neuber et al.2010. Eph receptors and Ephrin ligands: important players in angiogenesis and tumour angiogenesis. Journal of Oncology. Published on line March 10 doi: p135285.*

3. *P.Schneider, I. Dubus, F .Gouel, E. Legrand. 2011. What a role of angiogenesis in childhood ALL. Hema J November, doi:PMC 3216383*

4. *Weidner N, Semple JP, Welsch WR, Folkman J. 1991. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med; P324:1-8.*

5. *ChanchalRana, Seema Sharma,Vinita Agrawal and Uttam Singh (2010). Bone marrow angiogenesis in multiple myeloma and its correlation with clinicopathological factors. Ann Hematolo Art;Vol 89: p 789-794.*

6. *Thomas DA, Giles FJ, Cortes J, et al.Antiangiogenic therapy in leukemia.Acta Haematol. 2001; 106:190-207.*

7. *.Perez-Atayde AR, Sallan SE, Tedrow U, Connors S, Allred E, Folkman J. 1997. Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. Am J Pathol; 150**:** 815-821. (IVSL)*

8. *144. Naoko Okumura, Hitomi Yoshida,et al.2012.P13K/AKT/PETN signaling as a molecular target in leukemia angiogenesis. Adv Hematol.28; p843085.*

9. *Moehler TM, Ho AD, Goldschmidt H, et al. 2003. Angiogenesis in hematologic malignancies. Critical Reviews in Oncology/Hematology; 45: 227-244.*

10. *Stefan Faderl, Kim-AnhDo, Marcella M, et al. 2005. Angiogenic factors may have adifferent prognostic role in adult acute lymphoplastic leukemia. Blood Journal. Vol 106; doi: 10.1182; p303-4307. (IVSL)*

11. *D.Ribatti, A.Vacca.2008. Angiogenesis and antiangiogenesis in hematological diseases. Magazine of European Medical oncology.Volume1, issue1, pp31-33.*

12. *Ali Mohammad Jawad, Batool A, Gh. Yassin. 2003. Adults Acute Lymphoplastic Leukemia (ALL) Criteria of patients with failed initial induction chemotherapy. Baghdad, J Fac Med; 45. No, 3-4: 261.*

13. *Dieter Hoelzer, Nicola Gokbuget, Oliver Ottman.et al. 2002. Acute lymphoplastic leukemia. In American Society of Hematology Education program Book, American Society of Hematology. p 167-8.*

14. *149. Ching-Hon pui,Leslie L Robison,A Thomas Look. 2008. Acute ymphoplastic leukemia. In American Society of Hematology Education program Book,Hematology; Vol 371: p 1030-43. (IVSL)*

15. *150. Nicola G0kbuget and Dieter Hoelzer.Adults acute lymphoplastic leukemia (ch24). A.VictorHoffbrand, DanielCatovsky, EdwardG.D. 2011. Postgraguate Hematology. 6th edition. Londonand Cambridges, AVH, DC, EGDT, ARG. p442.*

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