# Lactate Dehydrogenase, not Vascular Endothelial Growth Factor or Basic Fibroblast Growth Factor, Positively Correlates to Bone Marrow Vascularity in Acute Myeloid Leukemia

Chieh-Lin Teng[1,2], Ji-Hsiung Young[1], Shih-Lan Hsu[3], Guan Chou[4], Ing-Tiau Kuo[5], Chen-Yuan Yu[3], Guang-Yuh Hwang[2]*

*[1]Division of Hematology/Oncology, Department of Internal Medicine, [3]Department of Education and Medical Research, and [4]Department of Pathology, Taichung Veterans General Hospital, [2]Department of Life Sciences, Tunghai University, Taichung, and [5]Department of Education and Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, R.O.C.*

**Background:** Angiogenesis has been extensively studied in acute myeloid leukemia (AML). Lactate dehydrogenase (LDH), a common biochemical marker for tumor burden and anaerobic glycolysis, is a poor prognostic factor for AML. Regulated by hypoxia-induced factor, both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are responsive to cancer-related angiogenesis. To study the roles of serum LDH, VEGF and bFGF in AML angiogenesis, we investigated bone marrow vascularity in untreated AML patients, and analyzed its relationship to serum LDH, VEGF and bFGF levels.

**Methods:** Eighteen (11 males, 7 females; mean age, 57.7 years) *de novo*, untreated AML patients were enrolled. Bone marrow vascularity was evaluated by staining bone marrow core biopsy tissue with endothelial cell marker CD31 or CD34. Serum LDH was determined with the Wroblewski-La Due method. Serum VEGF and bFGF were determined with enzyme-linked immunoassay. The relationship of LDH, VEGF and bFGF level to bone marrow vessel numbers was examined by linear regression.

**Results:** Log LDH significantly correlated to AML bone marrow vascularity ($r = 0.61$; $p = 0.007$). VEGF and bFGF concentrations did not correlate with AML angiogenesis.

**Conclusion:** These results suggest that serum LDH, but not VEGF and bFGF concentrations, can be used as a simple parameter for predicting vessel formation in AML bone marrow. [*J Chin Med Assoc* 2006;69(11):534–537]

**Key Words:** acute myeloid leukemia, angiogenesis, basic fibroblast growth factor, lactate dehydrogenase, vascular endothelial growth factor

## Introduction

Angiogenesis is a complex, multistep process by which new microvessels are formed from preexisting vasculature.[1] Hypoxia is the factor most responsible for this process.[2] Increased anaerobic glycolysis due to elevated tumor cell proliferation and metabolism is one of the most important physiologic responses to hypoxia. When tissue undergoes anaerobic glycolysis, lactate dehydrogenase (LDH) increases to accelerate conversion of pyruvate to lactate, yielding energy.[3] At the molecular level, hypoxia induces expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) for angiogenesis.[2] Increased angiogenesis has been observed in acute myeloid leukemia (AML) patients.[4] Simple parameters for prediction of angiogenetic ability in AML remain unclear. We investigated the association of peripheral blood serum LDH, VEGF and bFGF with bone marrow vascularity in AML patients.

*Correspondence to: Professor Guang-Yuh Hwang, Department of Life Sciences, Tunghai University, 181, Section 3, Chungkang Road, Taichung 407, Taiwan, R.O.C.
E-mail: gyhwang@thu.edu.tw • Received: April 3, 2006 • Accepted: September 22, 2006

## Methods

### *Study subjects*

Study subjects were 18 untreated, *de novo* AML patients (11 males, 7 females; mean age, 57.7 ± 18.2 years). The diagnosis of AML was determined according to criteria proposed by the French-American-British Cooperative Group in 1984.[5] Patient enrollment was voluntary and with informed consent. This project was approved by the institutional review board of Taichung Veterans General Hospital (No. 940103/453).

### *Bone marrow vessel counting*

After staining bone marrow core biopsy tissue with endothelial cell marker CD31 (1:1,600; Dako Inc., Carpinteria, CA, USA) or CD34 (1:500; Vector Laboratories Inc., Burlingame, CA, USA) (Figure 1), 2 of the authors separately assessed vessel number in each bone marrow biopsy slide with light microscopy (400×) in 5 areas containing the highest numbers of vessels, as described by Padro et al.[4]

### *LDH, VEGF and bFGF assays*

All assays were determined in duplicate; the mean value was used in statistical analysis. LDH in blood sera was determined with the Wroblewski-La Due method kit (Wako Ltd., Osaka, Japan). VEGF and bFGF in blood sera were determined with quantitative sandwich enzyme human VEGF and bFGF immunoassay kits (R&D Systems Inc., Minneapolis, MN, USA). LDH levels are presented in IU/L, and VEGF and bFGF concentrations in pg/mL. The minimum detection limits for VEGF and bFGF were 9 pg/mL and 3 pg/mL, respectively.

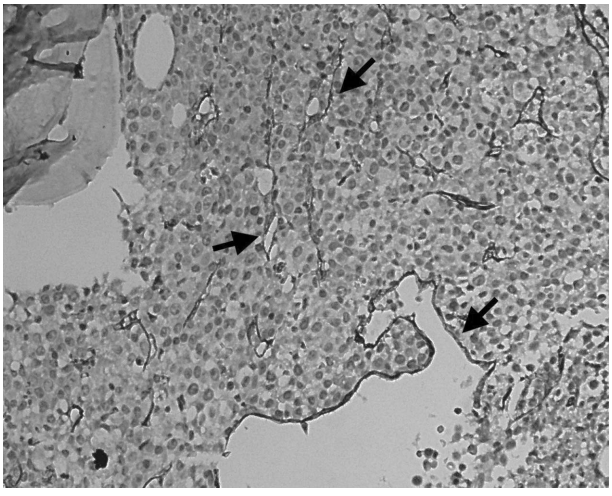

**Figure 1.** Immunohistochemical stain of bone marrow biopsy with CD31 showing brown-colored endothelial cells with vessel structure (arrows) (immunohistochemical stain with CD31, 400×).

### *Statistical analysis*

The relationship of LDH, VEGF and bFGF concentrations to bone marrow vessel numbers and correlation among LDH, VEGF and bFGF were examined by linear regression. LDH was log transformed to improve similarity to normal distribution. Statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Significance was set at $p < 0.05$.

## Results

Patients' basic data and original experimental results, including bone marrow vascularity, LDH level, VEGF and bFGF concentrations, are shown in Table 1. Vessel numbers in AML patients' bone marrow tissue were positively correlated to log peripheral blood serum LDH (Pearson's correlation coefficient, $r = 0.61$; $p = 0.007$) (Figure 2). VEGF data were not available for 2 patients due to insufficient specimens. Peripheral blood serum bFGF could not be detected in most of the patients. Bone marrow vascularity did not correlate to either peripheral blood serum VEGF or bFGF concentrations (VEGF: $r = 0.44$, $p = 0.86$; bFGF: $r = 0.22$, $p = 0.39$). There were no correlations among LDH, VEGF and bFGF concentrations.

## Discussion

Angiogenesis has been extensively studied in AML,[6] but simple parameters for predicting degree of AML angiogenesis are still absent. Our study demonstrated that serum LDH level is positively correlated to bone marrow vascularity in AML.

High LDH is a poor prognostic factor for several malignancies, including AML.[7] Some possible mechanisms have been proposed. First, acidification of the extracellular water space by lactate and the subsequent activation of tumor invasion may be a rational explanation.[8] Second, low pH microenvironment may increase cancer cell resistance to hypoxia-induced apoptosis by protecting mitochondria from oxidative stress.[9,10] And third, overexpression of LDH, especially LDH5, reflects an upregulated hypoxia-induced factor pathway, which regulates glycolysis, angiogenesis, resistance to apoptosis, and even cancer metastasis.[11–13]

Due to difficulty in counting leukemic tumor burden by tumor size or number of tumor cells, LDH is viewed as a simple biochemical marker for leukemic tumor burden prediction. Furthermore, Vidriales et al[14] showed that serum LDH level was proportional to leukemic cells in synthetic phase. Also, bone marrow

**Table 1.** Patients' basic data and original experimental results

| Patient | FAB classification | Sex | Age (yr) | Vessel no. | LDH (IU/L) | VEGF (pg/mL) | bFGF (pg/mL) |
|---|---|---|---|---|---|---|---|
| 1 | M3 | M | 29 | 37 | 617 | 84.8 | <3 |
| 2 | M0 | F | 36 | 19 | 269 | 80.2 | <3 |
| 3 | M2 | F | 50 | 9 | 373 | 38.7 | <3 |
| 4 | M2 | M | 66 | 18 | 172 | 127.3 | <3 |
| 5 | M2 | M | 69 | 21 | 403 | 134.6 | <3 |
| 6 | M2 | M | 70 | 28 | 832 | 101.1 | <3 |
| 7 | M2 | M | 29 | 24 | 302 | 91.6 | 4.2 |
| 8 | M3 | F | 49 | 38 | 429 | 150.4 | <3 |
| 9 | M4 | F | 76 | 57 | 352 | N/A | <3 |
| 10 | M2 | M | 74 | 18 | 323 | 206.6 | <3 |
| 11 | M3 | M | 38 | 28 | 1,028 | 91.3 | <3 |
| 12 | M1 | F | 49 | 37 | 260 | 158.8 | 4.6 |
| 13 | M4 | M | 75 | 20 | 474 | 158.1 | 3.5 |
| 14 | M3 | M | 42 | 51 | 1,689 | 988.2 | 6.0 |
| 15 | M2 | F | 82 | 30 | 929 | N/A | <3 |
| 16 | M4 | F | 58 | 55 | 4,621 | 214.1 | <3 |
| 17 | M4 | M | 61 | 25 | 321 | 46.4 | <3 |
| 18 | M2 | M | 86 | 19 | 363 | 538.2 | <3 |

*FAB = French-American-British; LDH = lactate dehydrogenase; VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor; N/A = not available.*

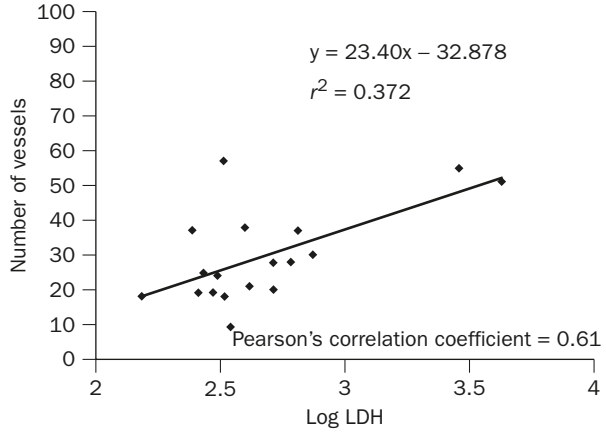


**Figure 2.** The number of vessels in acute myeloid leukemia bone marrow tissue is positively correlated to the log of blood serum lactate dehydrogenase activity (Log LDH) ($p = 0.007$).

microenvironment plays an important role in elevated LDH in AML. Compared to other solid malignancies, AML obviously has a higher cell proliferation rate. Under rapid proliferation and immaturity of tumor cells, LDH is released due to multiple cytokine activity and cell membrane damage.[15] The correlation between serum LDH and vascularity in AML bone marrow may reflect not only the degree of microenvironmental hypoxia, but also the degree of marrow involvement with leukemic cells, the aggressiveness of the leukemia, or the involvement of various cytokines.

As previously mentioned, LDH reflects an upregulated hypoxia-induced factor pathway. VEGF and bFGF, positively regulated by hypoxia-induced factor, were also surveyed in this study. The roles of VEGF and bFGF in AML angiogenesis remain controversial. The importance of both VEGF and bFGF in AML angiogenesis was reported,[16,17] but it has also been proposed that both VEGF and bFGF concentrations in AML patients are similar to those in healthy people.[17,18] VEGF receptors, rather than VEGF itself, have prognostic value.[18] A study by Bieker et al[16] demonstrated that degree of bFGF expression in AML did not correlate with microvessel density. Our study confirmed their results, and further demonstrated the lack of correlation between serum VEGF level and AML bone marrow vascularity. Serum VEGF and bFGF were not good molecular markers of angiogenesis in AML.

VEGF and bFGF are currently known to be growth factors for hematopoietic cells, and not just angiogenic factors.[19,20] The local effects of VEGF and bFGF on leukemic cell proliferation or angiogenesis may not be reflected in their peripheral blood concentrations. Further studies are required to answer this question.

We found a positive correlation between serum LDH level and bone marrow vascularity in AML patients, but no correlation of serum VEGF and bFGF levels with AML angiogenesis. These results suggest that serum LDH, but not VEGF and bFGF concentrations, can be used as a simple parameter in the evaluation of the angiogenic ability of AML. Anti-angiogenesis therapy is now rapidly developing in cancer treatment. Our findings may establish a simple indicator and

rationale that AML patients with high serum LDH level may be candidates for anti-angiogenesis treatment.

## Acknowledgments

We thank Taichung Veterans General Hospital for supporting the research (TCVGH-943701A) and statistical consultation.

## References

1. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003;9:653–60.
2. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 2003;9:677–84.
3. Walenta S, Mueller-Klieser WF. Lactate: mirror and motor of tumor malignancy. *Semin Radiat Oncol* 2004;14:267–74.
4. Padro T, Ruiz S, Bieker R, Burger H, Steins M, Kienast J, Buchner T, et al. Increased angiogenesis in the bone marrow of patients with acute myeloid leukemia. *Blood* 2000;95:2637–44.
5. Bernard P, Reiffers J, Lacombe F, Dachary D, Boisseau MR, Broustet A. A stage classification for prognosis in adult acute myelogenous leukaemia based upon patients' age, bone marrow karyotype and clinical features. *Scand J Haematol* 1984; 32:429–40.
6. Hussong JW, Rodgers GM, Shami PJ. Evidence of increased angiogenesis in patients with acute myeloid leukemia. *Blood* 2000;95:309–13.
7. Krykowski E, Polkowska-Kulesza E, Robak T, Matusewicz W, Urbanska-Rys H, Holub A. Analysis of prognostic factors in acute leukemias in adults. *Haematol Blood Transfus* 1987;30: 369–72.
8. Stubbs M, McSheehy PM, Griffiths JR, Bashford CL. Causes and consequences of tumour acidity and implications for treatment. *Mol Med Today* 2000;6:15–9.
9. Bronk SF, Gores GJ. Acidosis protects against lethal oxidative injury of liver sinusoidal endothelial cells. *Hepatology* 1991;14: 150–7.
10. Nemoto S, Takeda K, Yu ZX, Ferrans VJ, Finkel T. Role for mitochondrial oxidants as regulators of cellular metabolism. *Mol Cell Biol* 2000;20:7311–8.
11. Firth JD, Ebert BL, Ratcliffe PJ. Hypoxic regulation of lactate dehydrogenase A: interaction between hypoxia-inducible factor 1 and cAMP response elements. *J Biol Chem* 1995;270:21021–7.
12. Koukourakis MI, Giatromanolaki A, Simopoulos C, Polychronidis A, Sivridis E. Lactate dehydrogenase 5 (LDH5) relates to up-regulated hypoxia-inducible factor pathway and metastasis in colorectal cancer. *Clin Exp Metastas* 2005;22: 25–30.
13. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* 1996;271:32529–37.
14. Vidriales MB, Orfao A, Lopez-Berges MC, Gonzalez M, Lopez-Macedo A, Ciudad J, Lopez A, et al. Prognostic value of S-phase cells in AML patients. *Br J Haematol* 1995;89:342–8.
15. Jurisic V, Kraguljac N, Konjevic G, Spuzic I. TNF-alpha induced changes in cell membrane antigen expression on K-562 cells associated with increased lactate dehydrogenase (LDH) release. *Neoplasma* 2005;52:25–31.
16. Bieker R, Padro T, Kramer J, Steins M, Kessler T, Retzlaff S, Herrera F, et al. Overexpression of basic fibroblast growth factor and autocrine stimulation in acute myeloid leukemia. *Cancer Res* 2003;63:7241–6.
17. Litwin C, Leong KG, Zapf R, Sutherland H, Naiman SC, Karsan A. Role of the microenvironment in promoting angiogenesis in acute myeloid leukemia. *Am J Hematol* 2002;70:22–30.
18. Gwang Kim J, Kyun Sohn S, Hwan Kim D, Ho Baek J, Young Lee N, Soo Suh J, Chae SC, et al. Clinical implications of angiogenic factors in patients with acute or chronic leukemia: Hepatocyte growth factor levels have prognostic impact, especially in patients with acute myeloid leukemia. *Leuk Lymphoma* 2005;46:885–91.
19. Fiedler W, Graeven U, Ergun S, Verago S, Kilic N, Stockschlader M, Hossfeld DK. Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. *Blood* 1997;89:1870–5.
20. Kashiwakura I, Takahashi TA. Fibroblast growth factor and *ex vivo* expansion of hematopoietic progenitor cells. *Leuk Lymphoma* 2005;46:329–33.