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ORIGINAL ARTICLE

Clinical relevance of angiopoietin-1, angiopoietin-2, and their receptor Tie-2 expression in acute myeloid leukemia

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Abstract The angiogenic-related factors, angiopoietin-1 and angiopoietin-2 and their receptor Tie-2, have wide-ranging effects on tumor behavior that includes angiogenesis and inflammation. These multifaceted pathways present a potential target in developing novel inhibition strategies for cancer therapy. The present work aimed at detecting the prevalence of expression of angiopoietin-1, angiopoietin-2, and their receptor Tie-2 in 56 Egyptian de novo acute myeloid leukemia (AML) patients by conventional RT-PCR to verify the prognostic impact of their expression on the response to induction chemotherapy. Thirty age- and sex-matched healthy volunteers were subjected to the same analysis as a control group. High expression of Ang-1 was detected in the patient group but not the control group. AML patients expressing Ang-2 either solely or in combination with high Ang-1 and/or Tie-2 showed unfavorable response to induction chemotherapy, either failed induction or death during induction. These data provide evidence that the alternation of angiopoietin balance in favor of Ang-2 may play a critical role in the pathophysiology of AML. Furthermore, positive pretherapeutic expression of Ang-2 indicates viable unfavorable prognostic marker in AML patients and may be used as a prognostic tool in the risk-adaptive management of AML.

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Introduction

Angiogenesis is defined as the production of new blood vessels from an existing vascular network. It is a complex, tightly regulated multistep process, which includes activation of existing endothelial cells, degradation of the extra cellular matrix, proliferation, and migration of endothelial cells towards the angiogenic stimulus (Giles 2001).

Angiogenic growth factors that stimulate angiogenesis can be classified as direct angiogens, indirect angiogens, and angiogenic molecules. Direct angiogens include vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and hepatocyte growth factor (Ferrara and Alitalo 1999). Indirect angiogens, whose vascular effects are derived from the paracrine stimulation of direct angiogenic molecules, include interleukin-6, tumor necrosis factor-α, platelet-derived growth factor, and transforming growth factor-β (Giles 2001). Angiogenic molecules include angiopoietins, angiogenin, and angiotropin. The angiopoietin family consists of Ang-1, Ang-2, Ang-3, and Ang-4 (Lee et al. 2004). Of them, Ang-1 and Ang-2 are well characterized (Fiedler and Augustin 2006).

Ang-1 binds to and activates the receptor tyrosine kinase Tie-2. Ang-2 has similar affinity to Tie-2 and competes with the binding of Ang-1 to Tie-2; therefore, Ang-1 and Ang-2 are believed to function as naturally occurring antagonists (Lee et al. 2007). The role of Ang-2 in angiogenesis is still controversial. In initial studies, Ang-2 has been shown to block the effect of Ang1 on endothelial cells in vitro. Ang-2 competes with Ang-1 for binding to their common receptor Tie-2 and acts as a competitive inhibitor of Ang-1 (Loges et

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al. 2005). Angiopoietin-1 and angiopoietin-2 and their receptor Tie-2 have wide-ranging effects on tumor behavior that include angiogenesis, inflammation, and vascular extravasations. These multifaceted pathways present a vascular opportunity in developing novel inhibition strategies for cancer therapy (Shim et al. 2007).

Marrow angiogenesis in acute myeloid leukemia (AML) is a complex process involving the interplay of different angiogenic growth factors. Until now, most investigators have attempted to elucidate the impact of a single angiogenic factor on the pathogenesis or the prognosis of hematological malignancies (Teng et al. 2006; de Bont et al. 2001; Verstovsek et al. 2002). However, because of the orchestrated action of various angiogenic cytokines, it is necessary to analyze simultaneously their expression in identical pretreatment samples to gain insights into their relative relevance for the disease process. The endothelium-specific growth factors of the VEGF and angiopoietin family are believed to represent the most specific inducers of angiogenesis. Several members of both families have been shown to be constitutively expressed by AML blasts (Muller et al. 2002; Watarai et al. 2002). Paracrine exchange of growth factors between AML blasts and endothelial cells is believed to contribute to the pathogenesis of AML (Loges et al. 2005).

In the present work, we aimed at detecting the frequency of expression of the angiogenic-related factors angiopoietin-1, angiopoietin-2, and their receptor Tie-2 in Egyptian de novo AML patients, and to verify the prognostic impact of their expression on the response to induction chemotherapy.

Subjects and methods

The present study included 56 newly diagnosed adult acute myeloid leukemia patients attending the Nuclear Medicine Department, Kasr El-Aini Teaching Hospital, Faculty of Medicine, Cairo University. They were 35 males and 21 females. Their ages have a mean value of 39.64 years. Thirty age- and sex-matched healthy volunteers were included in this study as a control group. Patients were diagnosed according to the French–American–British (FAB) cooperative group criteria (Bennett et al. 1985). All patients were investigated at presentation prior to therapy. Patients were subjected to the following:

- Full history taking and clinical examination with careful assessment of clinical signs relevant to AML as lymphadenopathy, hepatomegaly, splenomegaly, fever, fatigue, weight loss, jaundice, pallor, purpura, ecchymosis, easy bruising, recurrent infections, gum hyperplasia, and bone and joint pain.
- CT of chest, abdomen, and pelvis are done to assess lung, liver, spleen, lymph nodes, and kidneys for possible pathological alterations.
- Cardiac examination including echocardiography and ejection fraction to assess the cardiac condition of the patients that might be affected by anthracycline chemotherapy.
- Routine laboratory investigations as differential blood count, liver and kidney functions, serum uric acid, LDH, and coagulation profile are done.
- Bone marrow examination and cytochemical studies (including myeloperoxidase, non-specific esterase, and dual esterase reactions).
- Immunophenotyping was done to establish the FAB sub-typing.

Detection of Ang-1 and Ang-2 and Tie-2 expression by RT-PCR Five milliliter of blood was withdrawn from every patient as well as the healthy volunteers in a sterile EDTA vacutainer. The mononuclear cells are separated and preserved at −70°C. Total cellular RNA was extracted from the mononuclear cells using the QIA amp RNA blood Mini kit (QIAGEN, Catalogue number. 52304), followed by c-DNA preparation using Revert Aid^{TM} First strand cDNA synthesis kit (Fermentas, K1621). A volume of 5 μl cDNA was added to a final PCR reaction mixture of 25 μl containing 12.5 μl Master Mix (Fermentas K0171, which contains TaqDNA polymerase in reaction buffer, $MgCl_2$, and dNTPs) and 1 μl of 10 μM of each of the forward and reverse specific primers of Ang-1 or Ang-2 or Tie-2 with β-actin. β-Actin was amplified as an internal control to study the purity of the extracted RNA. The primer sequences as well as the thermo-cycler program for Ang-1 and Tie-2 expression was performed according to Muller et al. (2002), while Ang-2 gene expression was performed as described by Wakabayashi et al. (2004). The primer sequences of the studied genes and the thermo-cycler programs used to study their expression are presented in Tables 1 and 2.

The expression of Ang-1 in the samples was judged according to the number of PCR cycles applied for its amplification. For Ang-1 expression, all cases and control samples were studied initially at 35 PCR cycles. Positive cases were considered as low Ang-1 expressers. Absent PCR product at 35 PCR cycles was considered as negative expression. Positive cases were re-analyzed at 25 cycles. Positive cases at 25 cycles were considered as high Ang-1 expressers. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 261 bp for Angio-1 (Fig. 1), 236 bp for Angio-2 (Fig. 2), 413 bp for Tie-2 (Fig. 3).

Table 1 Ang-2, Ang-1, Tie-2, and β-actin specific primers sequence and the size of the PCR product

Gene	Primer sequence	PCR product (bp)
Ang-1	F: 5-AACGCTCTGCAGAGAGATGCTCCA-3′ R: 5′-CTGGGTCTCAACATCTGTCAGCTT-3′	261
Ang-2	F: 5′-CAG AGG CTG CAA GTG CTG GAG AACA-3′ R: 5′-GAG GGA GTG TTC CAA GAG CTG AAG T-3′	236
Tie-2	F: 5′-CAAACCCGTTAATCACTATGAGGC-3′ R: 5′-CCTTGGTGTTGACTCTAGCTCGG-3′	413
β-Actin	F: 5′-TGA GGG CTA CCC ACA TCG TGC CCA TCT A-3′ R: 5′-CTA GAA GAC TTT GCG GTG GAC GAT GGA GGG-3′	661

Assessment of the response to therapy Successful treatment of AML requires control of bone marrow, systemic disease, and specific treatment of central nervous system (CNS) disease, if present. The cornerstone of this strategy includes systemically administered combination chemotherapy. Because only 5% of patients with AML develop CNS disease, prophylactic treatment is not indicated. Treatment of AML is divided into two phases: induction (to attain remission) and post-remission (to maintain remission). Maintenance therapy for AML was previously administered for several years but is not included in most current treatment clinical trials (Cassileth et al. 1992).

Induction of remission Patients were subjected to seven to three protocol for induction of remission: Novantrone, 12 mg/m^2, IV on days 1 and 3; ARA-C, 100 mg/m^2, continuous IV infusion, from day l to 7. If remission is not achieved, this protocol was repeated again. If there is no or minimal response, patients were shifted to high-dose chemotherapy. Induction therapy for acute promyelocytic leukemia included oral administration of all-*trans*-retinoic acid (ATRA) 45 mg/m^2/day PO until complete remission (CR) induces remission in 70% to 90% of patients with M3 AML. ATRA induces terminal differentiation of the leukemic cells followed by restoration of nonclonal hematopoiesis. Patients failing to respond to one or two cycles of such treatment are considered refractory (Licht et al. 1995).

Patients were admitted in the inpatient unit, and they usually spend about 1 month in the hospital (MacCallum et al. 1995).

Consolidation High-dose ARA-C for four cycles; ARA-C, 2 g/m^2, over 2-h infusions, every 12 h on days 1–4 (Goldstone et al. 2001; Stone et al. 2001). CR status was defined by normalization of the neutrophil count (at least ≥1.5/μL) and platelet counts (>100×10^3/mm^3) and marrow aspirate and biopsy that demonstrate at least 20% cellularity, less than 5% blasts, and no Auer rods, as well as absence of extramedullary infiltration. In addition, no signs or symptoms are evident of central nervous system involvement. Because the vast majority of AML patients meeting these criteria for remission have residual leukemia, modifications to the definition of complete remission have been suggested, including cytogenetic remission, in which a previously abnormal karyotype reverts to normal, and molecular remission, in which interphase fluorescence in situ hybridization (FISH) or multiparameter flow cytometry are used to detect minimal residual disease. Immunophenotyping and interphase FISH have greater prognostic significance than the conventional criteria for remission (Bacher et al. 2006).

To study the possible association between the response to induction chemotherapy and the expression of these angiogenic-related factors, the response to therapy was classified according to De Greef et al. (2005) as follows: (1) CR: cellular marrow with blast cells ≤5%, peripheral blood picture shows a neutrophil count ≥1.5×10^3/ml, platelet count ≥100×10^3/ml, and no evidence of leukemia in other sites; (2) death during or after the first course of therapy with aplastic or hypocellular marrow; or (3) primary resistance: cellular marrow with >5% blasts or evidence of leukemia in other sites.

Statistical analysis of the results Data were analyzed using SPSS statistical package version 15. Numerical data were expressed as mean ± standard deviation (SD), median,

Table 2 The thermo-cycler programs used to amplify the studied genes

Gene	Denaturation (°C)	Annealing (°C)	Extension (°C)	Number of PCR cycles
Ang-1	94	58	72	25 cycles (high expression), 35 cycles (low expression)
Ang-2	94	64	72	30 cycles
Tie-2	94	58	72	35 cycles
β-actin	94	58	68	30 cycles

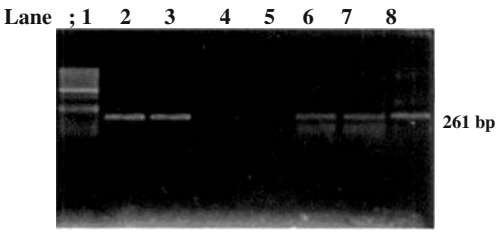

Fig. 1 Ang-1 mRNA expression by RT-PCR. *Lane 1* PCR marker (100 to 1,000 bp). *Lanes 2*, *3*, *6*, *7*, and *8* show positive Ang-1 expression. *Lane 4* and *5* are negative cases

minimum, and maximum. Qualitative data were expressed as frequency and percentage, as descriptive statistics. Comparison between qualitative variables (clinical data) was performed using the Chi-square test (Fisher's exact test), while for quantitative data (laboratory data), Mann–Whitney (non-parametric *t* test) was used. Comparison between groups was performed using Kruskal–Wallis (non-parametric ANOVA) test. For all statistical tests done, the threshold of significance is fixed at level 5%; *p* value less than 0.05 indicated a significant result.

Results

The current study was conducted on 56 newly diagnosed adult AML patients. They were 35 of 56 (62.5%) males and 21 of 56 (37.5%) females. Their ages have a mean value of 39.64 years. Thirty age- and sex-matched healthy volunteers were subjected to the same analysis as a control group. By conventional RT-PCR, mRNA expression of Ang-1, Ang-2, and Tie-2 genes in the patients as well as the control group is presented in Table 3.

Ang-1, Ang-2, and Tie-2 expression were significantly higher among AML patients (p=0.001, <0.001, and 0.01). High expression of Ang-1 was detected in the patient group but not the control group. All AML patients expressing high Ang-1 were Tie-2 positive, while five of them were also Ang-2 positive. No statistically significant difference

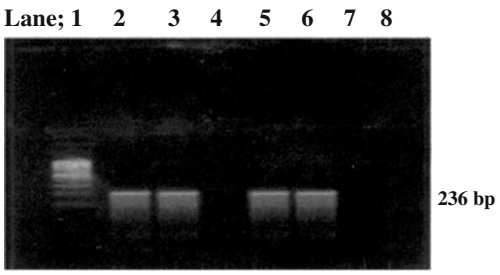

Fig. 2 Ang-2 mRNA expression by RT-PCR. *Lane 1* PCR marker (100 to 1,000 bp). *Lanes 2*, *3*, *5*, and *6* show positive Ang-2 mRNA expression. *Lane 4*, *6*, and *7* are negative cases

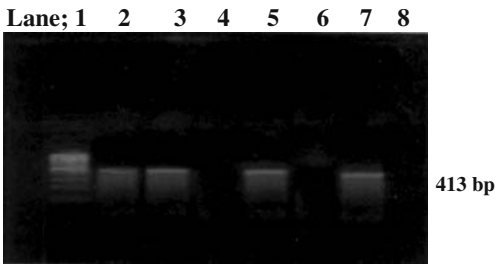

Fig. 3 Tie-2 mRNA expression by RT-PCR. *Lane 1* PCR marker (100 to 1,000 bp). *Lanes 2*, *3*, *5*, and *7* show positive Tie-2 mRNA expression. *Lanes 3*, *5*, and *8* are negative cases

noticed between high Ang-1 patients and the rest of patients as regards their age, gender, clinical data, laboratory data, FAB sub-typing, cytogenetic abnormalities, or response to induction therapy.

In this study, we focused on the influence of these angiogenic-related factors (Ang-2 expression and Ang-1 over expression; high Ang-1) on the response to induction chemotherapy in de novo AML patients. Accordingly, patients were sub-divided into three main groups according to their gene-expression pattern: group I, high Ang-1 positive/Ang-2 positive (H-Ang-1 +ve/Ang-2 +ve); group II, high Ang-1positive/Ang-2 negative (H-Ang-1 +ve/Ang-2 −ve); and group III, negative or low expression of Ang-1/Ang-2 positive (Ang-1 −ve/Ang-2 +ve).

Eight patients showed gene expression pattern similar to that of the control group, negative or low expression of Ang-1/Ang-2 negative (Ang-1 −ve/Ang-2 −ve). So, these cases were omitted from subgrouping of patients. That is why the three comparable subgroups included 48 patients.

Subgroup analysis revealed that there was no statistically significant difference encountered between the three studied groups as regards the clinical and laboratory data of the patients. However, AML patients expressing Ang-2 either solely or in combination with high Ang-1 and/or Tie-2 had unfavorable response to induction chemotherapy, either failed induction or death during induction as shown in Table 4.

As acute promyelocytic leukemia (APL–FAB-M3) has been considered as a separate disease entity among AML and the introduction of ATRA has dramatically improved its clinical outcome, we omitted FAB-M3 cases (six patients) from these groups and re-evaluated the response to therapy, but this did not affect the previous results obtained when M3 cases were included as shown in Table 5.

Discussion

The importance of angiogenesis for expansion of neoplastic diseases is a current problem in oncology. Emerging data

Table 3 Expression of Ang-1, Ang-2, and Tie-2 in AML patients and control group

Gene expression		Cases (number=56)		Control (number=30)		*p* value
Ang-1	Negative	17	30.3%	9	30%	0.001
	High	19	33.9%	0	0	
	low	20	35.7%	21	70%	
Ang-2	Negative	27	48.2%	28	93.3%	<0.001
	Positive	29	51.8%	2	6.67%	
Tie-2	Negative	20	35.7%	19	63.3%	0.01
	Positive	36	64.3%	11	36.67%	

suggest a critical role for bone marrow angiogenesis in hematologic malignancies. The angiopoietin/Tie ligand–receptor system is an essential regulator of this process (Kümpers et al. 2008). It is pointed to the importance of neovascularization in the pathogenesis of acute and chronic leukemias, lymphomas, and multiple myeloma. The knowledge of the importance of vascularization of neoplastic tissues is availing in therapy (researching of substances inhibiting angiogenesis), in diagnostics as a monitoring of a success of the therapy, and in prognosis (Grosicki et al. 2007).

In the current study, we aimed at detecting the expression of the angiogenic-related factors angiopoietin-1, angiopoietin-2, and their receptor Tie-2 in Egyptian de novo AML patients, and to verify the prognostic impact of their expression on the response to induction chemotherapy. Ang-1 was expressed in 39 of 56 (69.6%) of patients, 19 patients showed high expression of Ang-1, while 20 patients showed low Ang-1 expression. Ang-2 was expressed in 29 of 56 (51.7%) of patients, while Tie-2 was expressed in 36 of 56 (64.2%) of cases. This is in accordance with Watarai et al. (2002), Wakabayashi et al. (2004), Hatfield et al. (2008), Hou et al. (2008), and Bacher et al. (2006). Ang-1, Ang-2, and Tie-2 expression was significantly higher in the patient group compared to the control group. Similar results were reported by Hou et al. (2008), Loges et al. (2005), and Muller et al. (2002).

Table 4 Comparison between the three studied groups of de novo AML patients

Item		High Ang-1 +ve/Ang-2 +ve (number=5)	High Ang-1 +ve/Ang-2 −ve (number=19)	Ang-1–ve or low/Ang-2 +ve (number=24)	*p* value
Age (years)	Mean ± SD	41.8±14.9	46.1±16.5	35.1±16.4	0.09
Sex	Male	5	15	15	0.2
	Female	0	4	9	
Splenomegaly		1/5	8/19	6/24	0.4
Hepatomegaly		0/5	3/19	5/24	0.5
Lymphadenopathy		0/5	2/19	6/24	0.3
Bleeding tendency		3/5	7/19	13/24	0.4
Fever		2/5	8/19	16/24	0.2
Hb (g/dl)	Mean ± SD	9.4±1.4	7.7±2.2	8.0±1.6	0.2
	Range (median)	7.3–10.7 (10.2)	3.6–11 (7.3)	5.6–11 (7.8)	
WBCs $\times 10^3$/cm	Mean ± SD	46±45.5	32.5±28.7	72.3±135.4	0.4
	Range (median)	1.5–93.5 (37)	1.5–93.5 (28.8)	2.7–506.6 (36.7)	
Platelet $\times 10^3$/cm	Mean ± SD	59.4±64.8	51.7±44.1	36.2±22.5	0.3
	Range (median)	4–130 (22)	4–140 (30.2)	4–91 (32)	
PB blasts %	Mean ± SD	66.4±38.1	57.2±30.9	61.9±31.5	0.8
	Range (median)	13–100 (77)	11–100 (54)	12–100 (73.5)	
BM blasts %	Mean ± SD	92.8±16.1	75.2±27.6	69.9±24.7	0.2
	Range (median)	64–100 (100)	15–100 (80)	30–100 (71.5)	
Response to induction therapy	Favorable outcome	2/5	12/19	5/24	0.02
	Unfavorable outcome	3/5	7/19	19/24	
FAB classification	M1	2	6	8	0.5
	M2	0	3	8	
	M3	1	3	2	
	M4	2	6	5	
	M5	0	1	1	

Table 5 Statistical comparison between the different groups of non-M3 AML cases as regards their response to induction chemotherapy

Response to induction chemotherapy	High Ang-1 +ve/Ang-2 +ve (number=4)	High Ang-1 +ve/Ang-2 −ve (number=19)	Ang-1 –ve or low/Ang-2 +ve (number=24)	*p* value
Favorable outcome (CR)	2/4	10/16	5/22	0.004
Unfavorable outcome	2/4	6/16	17/22	

The expression of the genes under study was performed with conventional RT-PCR to detect the prevalence of these genes among Egyptian AML patients, and this technique is accurate, reliable, and cost effective especially as a screening test.

In the present work, high expression of Ang-1 was detected in the patient group but not the control group. All AML patients expressing high Ang-1 were Tie-2 positive. This is in accordance with the study of Muller et al. (2002), where all Ang-1 positive patients whether Ang-1 high or low expressed Tie-2. No statistically significant difference was noticed between high Ang-1 patients and the rest of patients as regards their age, gender, clinical data, laboratory data, FAB sub-typing, or cytogenetic abnormalities. This is in accordance with the studies of Lee et al. (2007), Loges et al. (2005), and Schlieman et al. (2006). However, Ang-1 expression was significantly higher in female patients (Schlieman et al. 2006). On the other hand, Muller et al. (2002) reported that high expression of Ang-1 was significantly associated with high peripheral blood blast count. Moreover, high expression of Ang-1 was significantly associated with unfavorable cytogenetic subtype (Lee et al. 2007).

Comparison between Ang-2 positive and negative cases revealed no statistically significant difference between them as regards their age, gender, clinical data, laboratory data, FAB sub-typing, or cytogenetic abnormalities. This is in agreement with the studies of Lee et al. (2007), Schlieman et al. (2006), and Loges et al. (2005).

Considering Tie-2 expression, comparison between Tie-2-positive and Tie-2-negative cases revealed no statistically significant difference as regards their age, gender, clinical data, laboratory data, FAB sub-typing, or cytogenetic abnormalities. This is in agreement with the study of Lee et al. (2007) and Hou et al. (2008), except for the sex difference as Tie-2 expression was significantly higher in male patients in the study of Lee et al. (2007). Furthermore, Watarai et al. (2002) reported that Tie-2 expression was significantly higher among FAB-M4 patients and negative in FAB-M3 patients.

In this study, we focused on the influence of these angiogenic-related factors (Ang-2 expression and Ang-1 over expression; high Ang-1) on the response to induction chemotherapy in de novo AML patients. Accordingly, patients were sub-divided into three main groups according to their gene-expression pattern. Group I, high Ang-1 positive/Ang-2 positive (H-Ang-1 +ve/Ang-2 +ve); group II, high Ang-1positive/Ang-2 negative (H-Ang-1 +ve/Ang-2 −ve); and group III, negative or low expression of Ang-1/Ang-2 positive (H-Ang-1 −ve/Ang-2 +ve).

Subgroup analysis revealed that there was no statistically significant difference encountered between the three studied groups as regards the clinical and laboratory data of the patients. However, AML patients expressing Ang-2 either solely or in combination with high Ang-1 and/or Tie-2 achieved unfavorable response to induction chemotherapy, either failed induction or death during induction. Similarly, Lee et al. (2007) and Hou et al. (2008) stated that expression of Ang-2 was a predictor of poor prognosis and poor overall survival in AML patients. Hou et al. (2008) reported that the effect of Ang-2 expression is significantly higher when coupled with low Ang-1, but not high Ang-1 expression. The reason that Ang-2 lost its impact on survival in AML patients with high Ang-1 was unclear. This may be due to the opposing actions of Ang-1 and Ang-2. Schlieman et al. (2007) estimated the plasma levels of circulating Ang-1, Ang-2, and soluble Tie-2 in AML patients and stated that high Ang-2 and Tie-2 levels were predictive of poor survival.

Oppositely, the study of Loges et al. (2005) revealed that higher Ang-2 mRNA levels in leukemic blasts indicate a better prognosis in AML patients. Moreover, Schlieman et al. (2006) discovered that AML patients expressing high levels of intracellular Ang-2 by immunostaining technique displayed a significantly extended overall survival, and Ang-2 is an independent prognostic factor for overall survival.

As angiogenesis is required for tumor formation and growth, inhibition of angiogenesis is a promising new approach in cancer therapy (Dong et al. 2008). Various angiogenesis inhibitors have been developed to target vascular endothelial cells and block tumor as in the in vitro studies and clinical trials of (UCN-01) on AML blasts (Sampath et al. 2006). Inhibitors of angiogenesis are antineoplastic drugs with relatively lower toxicity and lower risk of drug resistance than conventional chemotherapy, which is important especially during prolong administration, so they can be an alternative of therapeutic process (Grosicki et al. 2007).

On the basis of the findings obtained by this study, all patients with acute myeloid leukemia should be screened routinely before starting treatment for the concerted expression of angiopoietin-2, angiopoietin-1, and Tie-2 by conventional RT-PCR as Ang-2 may serve as a potential biomarker for prognosis being important for risk stratification in adult de novo AML. For Ang-2 positive cases, patients have to be followed by quantitative real-time PCR to monitor their response to therapy, detection of minimal residual disease, and early detection of relapse. Further investigations are indicated to understand how these angiogenic factors contribute to a more aggressive leukemia phenotype. Moreover, identification of these genes will pave the way to targeted treatment for patients with AML.

References

Bacher U, Kern W, Schoch C et al (2006) Evaluation of complete disease remission in acute myeloid leukemia: a prospective study based on cytomorphology, interphase fluorescence in situ hybridization, and immunophenotyping during follow-up in patients with acute myeloid leukemia. Cancer 106(4):839–847

Bennett JM, Catovsky D, Daniel MT et al (1985) Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 103(4):620–625

Cassileth PA, Lynch E, Hines JD et al (1992) Varying intensity of postremission therapy in acute myeloid leukemia. Blood 79 (8):1924–1930

de Bont ES, Rosati S, Jacobs S et al (2001) Increased bone marrow vascularization in patients with acute myeloid leukaemia: a possible role for vascular endothelial growth factor. Br J Haematol 113:296–304

De Greef G, Van W, Boogarts M (2005) criteria for defining a complete remission in acute myeloid leukemia. Br J Haematol 128(2):184

Dong D, Ni M, Li J et al (2008) Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. Cancer Res 68(2):498–505

Ferrara N, Alitalo K (1999) Clinical applications of angiogenic growth factors and their inhibitors. Nat Med 5(12):1359–1364

Fiedler U, Augustin HG (2006) Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol 27(12):552–558

Giles FJ (2001) The vascular endothelial growth factor (VEGF) signaling pathway: a therapeutic target in patients with hematologic malignancies. Oncologist 6(Suppl 5):32–39

Goldstone AH, Burnett AK, Wheatley K et al (2001) Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. Blood 98(5):1302–1311

Grosicki S, Grosicka A, Hołowiecki J (2007) Clinical importance of angiogenesis and angiogenic factors in oncohematology. Wiad Lek 60(1–2):39–46

Hatfield KJ, Hovland R, Øyan AM et al (2008) Release of angiopoietin-1 by primary human acute myelogenous leukemia cells is associated with mutations of nucleophosmin, increased by bone marrow stromal cells and possibly antagonized by high systemic angiopoietin-2 levels. Leukemia 22(2):287–293

Hou HA, Choua WC, Lin LI et al (2008) Expression of angiopoietins and vascular endothelial growth factors and their clinical significance in acute myeloid leukemia. Leuk Res 32:904–912

Kümpers P, Koenecke C, Hecker H et al (2008) Angiopoietin-2 predicts disease-free survival after allogeneic stem cell transplantation in patients with high-risk myeloid malignancies. Blood 112 (5):2139–2148

Lee KW, Lip GY, Blann AD (2004) Plasma angiopoietin-1, angiopoietin-2, angiopoietin receptor tie-2, and vascular endothelial growth factor levels in acute coronary syndromes. Circulation 110(16):2355–2360

Lee CY, Tien HF, Hu CY et al (2007) Marrow angiogenesis-associated factors as prognostic biomarkers in patients with acute myelogenous leukaemia. Br J Cancer 97(7):877–882

Licht JD, Chomienne C, Goy A et al (1995) Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood 85 (4):1083–1094

Loges S, Heil G, Bruweleit M et al (2005) Analysis of concerted expression of angiogenic growth factors in acute myeloid leukemia: expression of angiopoietin-2 represents an independent prognostic factor for overall survival. J Clin Oncol 23(6):1109–1117

MacCallum PK, Rohatiner AZ, Davis CL et al (1995) Mitoxantrone and cytosine arabinoside as treatment for acute myeloblastic leukemia in older patients. Ann Hematol 71(1):35–39

Muller A, Lange K, Gaiser T et al (2002) Expression of angiopoietin-1 and its receptor TEK in hematopoietic cells from patients with myeloid leukemia. Leuk Res 26:163–168

Sampath D, Cortes J, Estrov Z et al (2006) Pharmacodynamics of cytarabine alone and in combination with 7-hydroxystaurosporine (UCN-01) in AML blasts in vitro and during a clinical trial. Blood 107(6):2517–2524

Schliemann C, Bieker R, Padro T et al (2006) Expression of angiopoietins and their receptor Tie2 in the bone marrow of patients with acute myeloid leukemia. Haematologica 91 (9):1203–1211

Schliemann C, Bieker R, Thoennissen N et al (2007) Circulating angiopoietin-2 is a strong prognostic factor in acute myeloid leukemia. Leukemia 21(9):1901–1906

Shim WS, Ho IA, Wong PE (2007) Angiopoietin: a TIE(d) balance in tumor angiogenesis. Mol Cancer Res 5(7):655–665

Stone RM, Berg DT, George SL et al (2001) Postremission therapy in older patients with de novo acute myeloid leukemia: a randomized trial comparing mitoxantrone and intermediate-dose cytarabine with standard-dose cytarabine. Blood 98(3):548–553

Teng CL, Young JH, Hsu SL et al (2006) Lactate dehydrogenase, not vascular endothelial growth factor or basic fibroblast growth factor, positively correlates to bone marrow vascularity in acute myeloid leukemia. J Chin Med Assoc 69(11):534–537

Verstovsek S, Kantarjian H, Manshouri T et al (2002) Prognostic significance of cellular vascular endothelial growth factor expression in chronic phase chronic myeloid leukemia. Blood 99:2265–2267

Wakabayashi M, Miwa H, Shikami M et al (2004) Autocrine pathway of angiopoietins-Tie2 system in AML cells; association with phosphatidyl-inositol 3 kinase. Hematol J 5:353–360

Watarai M, Miwa H, Shikami M et al (2002) Expression of endothelial cell-associated molecules in AML cells. Leukemia 16:112–119