# Changes in magnetic resonance bone marrow angiogenesis on day 7 after induction chemotherapy can predict outcome of acute myeloid leukemia

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## ABSTRACT

Recently, dynamic contrast-enhanced magnetic resonance imaging has been shown to be a non-invasive technique that provides global and functional imaging of bone marrow angiogenesis in acute myeloid leukemia.

To assess the clinical implication of changes in angiogenesis shortly after induction chemotherapy, dynamic contrast-enhanced magnetic resonance imaging was performed prospectively before treatment (day 0) and on day 7 in 80 patients with *de novo* acute myeloid leukemia.

We demonstrated that a post-therapeutic reduction in *Peak* (negative Δ*Peak*) compared with the day 0 value was significantly associated with a higher chance of achieving complete remission, and better overall and disease free survival ($P$=0.022, 0.003 and 0.007, respectively). Cox's multivariate analysis also identified negative Δ*Peak* value as an independent good prognostic factor for overall and disease free survival. Our findings provide evidence that the change of *Peak* on day 7 relative to pre-treatment levels may be a relevant biomarker for early identification of patients who may fail conventional induction chemotherapy *(ClinicalTrials.gov Identifier: NCT00172562)*.

Key words: acute myeloid leukemia; BM angiogenesis, outcome, DCE-MRI.

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## Introduction

There is emerging evidence to show that angiogenesis plays a crucial role not only in solid cancers but also in hematologic malignancies.[1-3] Tumor angiogenesis in acute meyloid leukemia (AML) is traditionally determined by micro-vessel density (MVD) of the bone marrow (BM) by immuno-histochemical staining.[2-4] However, this method can only measure bone marrow angiogenesis in a very limited area, which is not representative of global or *in vivo* tumor angiogenesis and may not be easily interpreted on the background of post-therapeutic BM hypo-cellularity. Recently, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has been designed to measure global and functional bone marrow angiogenesis *in vivo*. It provides a non-invasive and reproducible method for direct quantification of blood vessel density, vascular flow, and permeability.[5,6]

Current risk-adapted therapy for AML patients is based on several important prognostic markers, such as age, WBC counts, cytogenetics, and genetic mutations at diagnosis.[7-9] There have been attempts to identify AML patients with poor response at an earlier stage of treatment in order to adopt new therapeutic strategies. Early quantitative assessments of post-chemotherapy cellularity, extent of cyto-reduction, and MVD change in BM biopsy specimens of AML patients have been previously reported;[10-14] however, the prognostic implications of these factors in AML patients remain unclear.

A previous work has shown that increased bone marrow angiogenesis measured by DCE-MRI at diagnosis can independently predict adverse clinical outcome in AML.[5,15] This prospective study sought to determine the change of BM angiogenesis by DCE-MRI on day 7 of induction chemotherapy in AML patients and evaluate its clinical implication. To the best of our knowledge, this is the first study of early assessment of dynamic BM angiogenesis after chemotherapy in AML patients. The changes in BM angiogenesis can be

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used as an early biomarker for assessing treatment response and for guiding further therapy.

## Design and Methods

### Patients

From November 2004 to March 2007, 80 consecutive patients with newly diagnosed *de novo* AML at the National Taiwan University Hospital were prospectively enrolled. All patients with non-M3 subtypes of AML were treated with standard induction chemotherapy (idarubicin 12 mg/m$^2$/d on days 1-3 and cytarabine 100 mg/m$^2$/d on days 1-7). After achieving complete remission, the patients then received consolidation therapy with a total of eight doses of high-dose cytarabine (2,000 mg/m$^2$ q12h, days 1-4) with or without an anthracycline. Patients with acute promyelocytic leukemia received concurrent all-trans retinoic acid and chemotherapy. DCE-MRI was performed at diagnosis before treatment (day 0 MRI) and on day 7 after induction chemotherapy (day 7 MRI). Every patient was followed-up until March 31, 2008.

### Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) of bone marrow

DCE-MRI protocols and methods were as previously described.[5,15] Briefly, MR imaging of the bone marrow was performed with a 1.5 Tesla superconducting system (Sonata; Siemens, Erlangen, Germany) at the midsection of the vertebral bodies from T11 to the sacrum. A turbo-fast low-angle shot gradient-echo pulse sequence was used and acquisition time was 2.0 second per frame contiguously and 300 dynamic images were obtained. An injection of gadopentetate dimeglumine containing 0.15mmol/KgBw of gadolinium was administered constantly (2.0mL/sec), immediately followed by a 20mL saline flush. Perfusion parameters, including the peak enhancement ratio (*Peak*), vascularity parameter amplitude (*Amp*), and permeability parameter (*K trans*) were calculated quantitatively from the time-intensity curve according to the bi-compartmental model.[16-18] *Peak* is defined as [SI (max) – SI (base) at the first pass)] / SI (base) and represents perfusion and contrast in intravascular and interstitial space. *Amp* represents plasma concentration and *K* trans means the exchange rate constant between extra-vascular extracellular space and the plasma. Furthermore, a color-coded map of DCE-MRI parameters was developed to illustrate the anatomic and functional information by incorporating conventional MR images.

### Statistical analysis

Overall survival (OS) was measured from the date of first diagnosis to the date of last follow up or death from any cause while disease free status indicated that the patient achieved complete remission and had not relapsed during the study period. Pre-treated (day 0) and day 7 angiogenesis parameters were compared using the paired t-test. Because age and sex factors may influence BM perfusion,[19] the implications of differences (day 7-day 0 Δvalue: Δ*Peak*, Δ*Amp* and Δ*K* trans) on clinical outcomes were investigated by Cox's regression with covariate adjustment. The impact of day 7 angiogenesis on survival was also analyzed using Cox's regression by adjusting for covariates. Cox's regression adjusted survival curves were used to plot survival curves, and two-sided -2log-likelihood [-2log(L)] tests were used to test the differences between groups. Moreover, multivariate Cox's regression analysis was adopted to estimate the hazard ratio of risk parameters by adjusting the effects of potential confounding variables. Angiogenesis parameters, age, sex, WBC count, lactate dehydrogenase (LDH) and karyotype were used as covariates. Data were analyzed using STATISTICA Data Miner software (version 8.0; StatSoft Inc, Tulsa, OK, USA) and SPSS software (release 15; SPSS Inc, Chicago, Illinois, USA).

**Table 1.** Multivariate analysis (Cox's regression) of the disease free survival and overall survival.

| Variable | Disease free survival RR | 95% CI | *P* value | Overall survival RR | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| Total patients (n=80) | | | | | | |
| Sex | 0.749 | 0.353-1.592 | 0.453 | 0.541 | 0.229-1.281 | 0.163 |
| Age[@] | 1.670 | 0.804-3.468 | 0.169 | 3.882 | 1.587-9.500 | 0.003* |
| WBC[ψ] | 1.006 | 0.463-2.187 | 0.998 | 0.718 | 0.294-1.750 | 0.466 |
| LDH[§] | 0.666 | 0.237-1.868 | 0.439 | 0.544 | 0.165-1.794 | 0.317 |
| Karyotype[ε] | 2.493 | 1.113-5.581 | 0.026* | 2.860 | 1.191-6.868 | 0.019* |
| *ΔPeak*[‡] | 0.157 | 0.041-0.609 | 0.007* | 0.093 | 0.019-0.458 | 0.003* |
| Peak[†] (pre-treated) | 2.882 | 1.395-5.956 | 0.004* | 4.560 | 1.890-11.003 | 0.001* |
| Non-APL patients (n=74) | | | | | | |
| Sex | 0.849 | 0.396-1.818 | 0.673 | 0.538 | 0.229-1.267 | 0.156 |
| Age@ | 1.528 | 0.734-3.182 | 0.257 | 3.814 | 1.558-9.338 | 0.003* |
| WBC[ψ] | 0.984 | 0.454-2.135 | 0.968 | 0.720 | 0.296-1.754 | 0.470 |
| LDH[§] | 0.749 | 0.266-2.110 | 0.584 | 0.545 | 0.166-1.789 | 0.317 |
| Karyotype[ε] | 2.602 | 1.106-6.123 | 0.029* | 2.710 | 1.099-6.684 | 0.030* |
| *ΔPeak*[‡] | 0.140 | 0.029-0.681 | 0.015* | 0.105 | 0.021-0.528 | 0.006* |
| *Peak*[†] (pre-treated) | 2.819 | 1.363-5.834 | 0.005* | 4.442 | 1.831-10.778 | 0.001* |

*CI: confidence interval; RR: relative risk; *Relative risk that is statistically significant (P<0.05); [@]Age >50 years vs. <50 years; [ψ]WBC >50 K/μL vs. <50 K/μL at diagnosis; [§]LDH >920 U/L vs. <920 U/L at diagnosis; [ε]Incremental RR of karyotype is the value at risk from each grade change (unfavorable, intermediate and favorable cytogenetics); [‡]negative ΔPeak relative to positive* ΔPeak*; [†]high pre-treated* Peak *relative to low pre-treated* Peak.

## Results and Discussion

### *Bone marrow angiogenesis magnetic resonance imaging on day 7 predicts clinical outcome*

Among the 80 AML patients recruited, 39 were males and 41 were females (median age 49 years; range 17-76 years). Sixty-three patients (79%) achieved complete remission and 41 (51%) remained disease free during the study period with a median follow up of 18 months.

Patients who remained disease free had significantly lower *Peak* and *Amp* values on day 7 ($P$=0.002 and 0.009, respectively; *Online Supplementary Figure S1A* and *S1B*; *Online Supplementary Table S1*). There was no difference in age, sex, karyotype, hemogram, LDH value, follow-up duration or *K trans* between these two groups. The time-intensity curve and the color-coded angiogenesis map of the vertebral BM in 2 patients are shown in Figure 1.

The impact of day 7 angiogenesis parameters on survival was analyzed by Cox's regression with adjustment for age and sex. Patients with higher *Peak* or *Amp* on day 7 after induction chemotherapy had significantly poorer disease free survival (DFS) ($P$=0.002 and 0.003, respectively) and overall survival (OS) ($P$=0.001 and <0.001, respectively). However, K trans on day 7 showed no influence on DFS and OS.

### *Post-therapeutic changes of bone marrow angiogenesis as prognostic indicators*

The median of MR angiogenesis parameters *Amp* and *K trans* on day 7 after induction chemotherapy was significantly reduced compared to those at initial diagnosis (median 0.0765 *vs*. 0.1575 and 0.0009 *vs*. 0.0118; both $P$<0.0001; *Online Supplementary Figure S1E* and *S1F*). On the other hand, Peak varied greatly on day 7 with a median that was significantly higher than that at initial diagnosis (median 1.995 *vs*. 1.224; $P$<0.0001; *Online Supplementary Figure S1D*).

To evaluate the impact of the changes in angiogenesis values from day 0 to day 7 (day 7- day 0, as Δ*value*) the patients were divided into two groups. Patients with a decrease in angiogenesis values on day 7 compared to baseline values were assigned as the negative Δ*value* group and those with an increase in values as the positive Δ*value* group. AML patients with negative Δ*Peak* on day 7 had a higher chance of achieving complete remission and remaining disease free (86.7% *vs*. 70.8%; $P$=0.022 and 80.0% *vs*. 44.6%; $P$=0.0204, respectively). Kaplan-Meier survival curves showed that patients with negative Δ*Peak* had better OS and DFS than those with positive Δ*Peak* ($P$=0.007 and 0.003, respectively; Figure 2). In contrast, AML patients with positive Δ*K trans* had better OS and DFS ($P$=0.029 and 0.023, respectively). There was no influence of Δ*Amp* on survival.

### *Post-therapeutic* ΔPeak *as an independent prognostic factor*

Multivariate Cox's proportional hazards analysis identified negative Δ*Peak* as an independent good prognostic factor for OS and DFS (relative risk 0.093, $P$=0.003 and relative risk 0.157, $P$=0.007, respectively; Table 1) after discriminating from other covariates, including pre-treated Peak. The same was also true for patients with acute non–promyelocytic leukemia (relative risk 0.105, $P$=0.006 and relative risk 0.140, $P$=0.015, respectively; Table 1).

Few studies have emphasized the impact of therapy-induced changes in angiogenesis in AML via the micro-vessel density method.[2,13,14] Kuzu *et al.* showed that AML patients who responded to treatment had a significant decrease in blast counts, cellularity, and micro-vessel den-

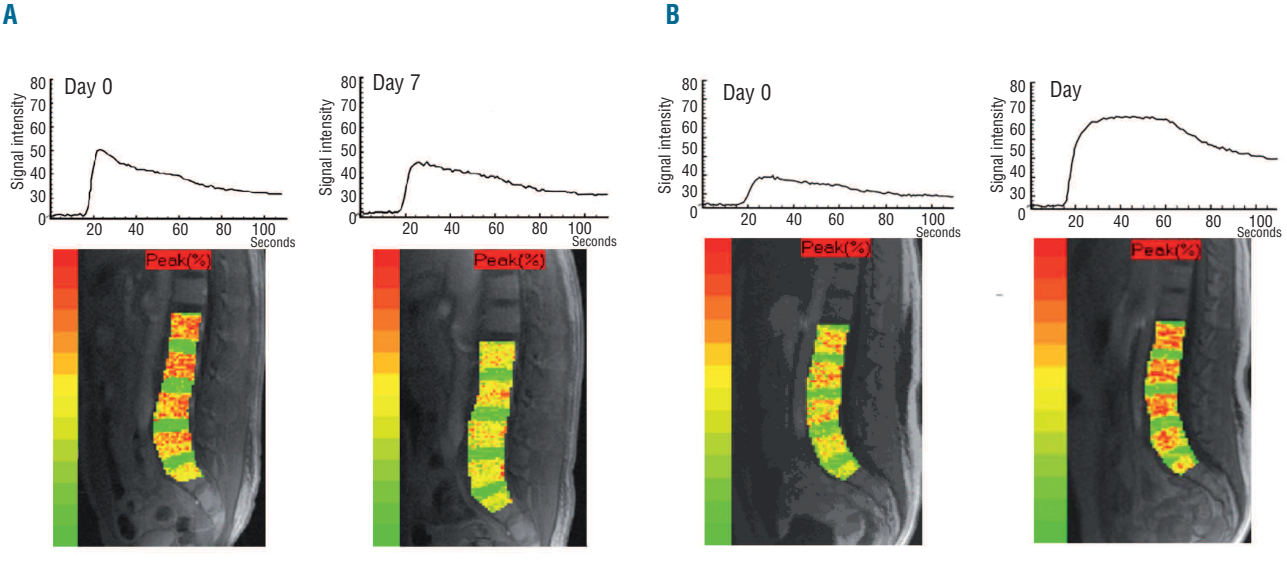


Figure 1. Representative time-intensity curves derived from DCE-MRI and color-coded angiogenesis maps of vertebral bone marrow of two patients. A 49-year old male AML patient achieved *Peak* reduction on day 7 (negative Δ*Peak*) after induction chemotherapy (A) and he still remained disease-free till the end of the study. In contrast, a 27-year old female patient with positive Δ*Peak* on day 7 DCE-MRI (B) relapsed 5.5 months after the achievement of first complete remission and died due to poor disease control. Color-coded angiogenesis map: red represents high angiogenesis, yellow intermediate and green low angiogenesis.

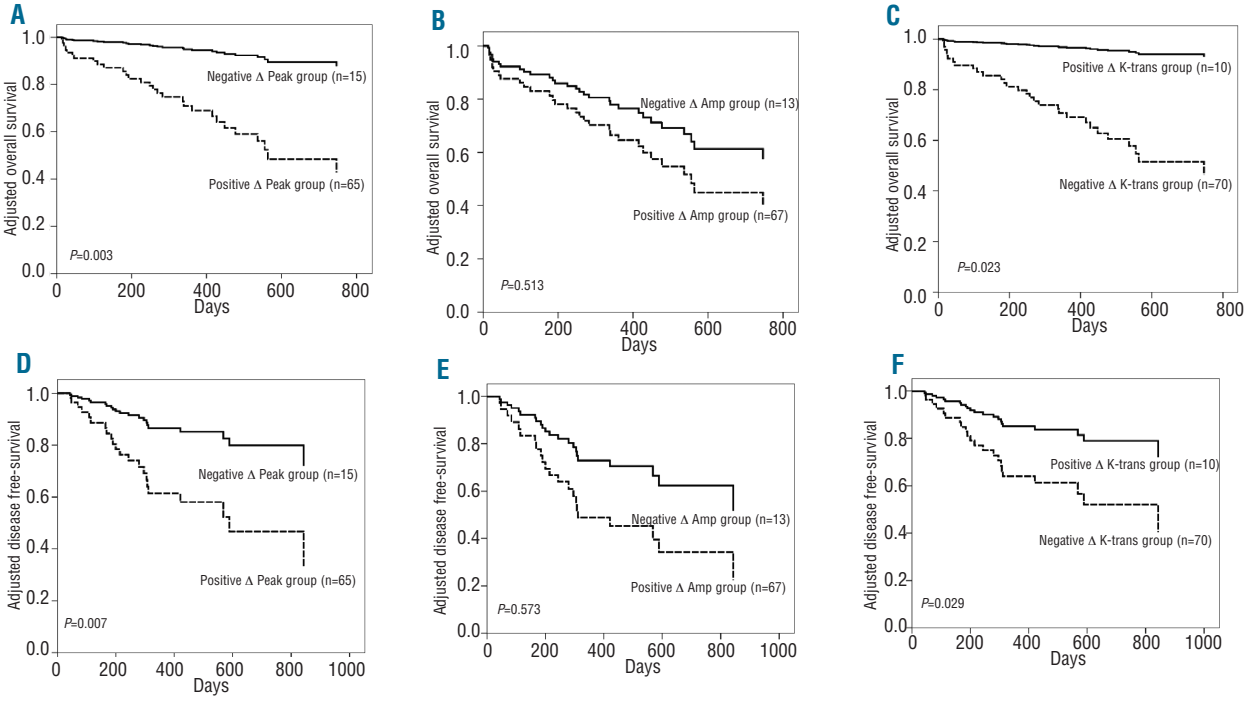


**Figure 2. Kaplan-Meier survival curves of overall and disease free survival in AML patients stratified by changes in angiogenesis parameters post-therapeutically. Patients with decreased *Peak* (negative Δ*Peak*) after chemotherapy (solid line) had significantly longer overall survival (*P*=0.003) (A) and disease free survival (*P*=0.007) (D) (positive Δ*Peak*, dashed line). In contrast, patients with increased K trans after chemotherapy (positive Δ*K trans*, solid line) had significantly longer overall survival (*P*=0.023) (C) and disease free survival (*P*=0.029) (F) than those with reduced K trans value (negative Δ*K trans*, dashed line). The change in Amplitude (Δ*Amp*) had no influence on overall and disease free survival (B and E).**

sity after induction chemotherapy and Rabitsch *et al.* demonstrated that BM MVD decreased in patients who responded to stem cell transplantation.[13,14] However, the impact of such a post-therapeutic change in MVD on clinical outcome was not clearly mentioned and the ideal time for early evaluation was unknown.

Early assessment of the BM after 4-10 days of induction chemotherapy in AML patients showed that the extent of cytoreduction or decrease in leukemia cells was associated with complete remission rate and remission duration.[11,20-22] Nevertheless, the number of patients studied is limited and it is not known whether findings of early BM assessment can independently predict survival in AML patients. This prospective study distinctly shows that DCE-MRI on day 7 and its dynamic changes can help distinguish AML patients who may remain disease free from those who probably will not. AML patients who achieve reduced Peak on day 7 (negative Δ*Peak*) have a significantly higher chance of achieving complete remission and longer OS and DFS, and Δ*Peak* is an independent prognostic factor. These findings suggest that Δ*Peak* may be used as an early biomarker for risk-adapted treatment. It is intriguing that *Peak* increases (positive Δ*Peak*) on day 7 in more than half of the patients and the reason of this is yet to be determined. It may reflect the effect of interaction among residual leukemia cells, chemotherapy-induced cytokine changes, and stromal cells.

In contrast, elevation of *K trans* on day 7 with respect to baseline value significantly correlates with prolonged OS and DFS. Although the reason for this phenomenon is not completely understood, it is hypothesized that the change in vascular permeability early after chemotherapy may play a role. In patients with increased *K trans* seven days post-chemotherapy, more chemotherapeutic agents may be delivered into the extravascular space leading to better cytotoxic effects. In multivariate analysis, however, Δ*K trans* is not an independent prognostic factor.

In the study of BM angiogenesis measured by DCE-MRI at a single time point, the confidence interval for each parameter may be influenced by the choice of quantification model, MR protocol, contrast injection rate, and different contrast agents. Thus, the results obtained in one study may not be applicable to another if each parameter is analyzed separately. However, the use of the "difference" in angiogenesis parameters from pre-treatment date to day 7 of chemotherapy, which in this study has been shown to be very useful in predicting treatment outcome, may be applied to any machine without requiring validation since it is the data between two time points obtained from patients using the same machine which are compared. Thus, analysis of DCE-MRI is reproducible and can achieve consensus in different research sites.

In summary, the use of the "difference" between pre-treatment and day 7 BM DCE-MRI provides a practical method for the early assessment of treatment response and predicting clinical outcome in AML patients. Increased BM perfusion on day 7 as reflected by positive Δ*Peak* value can independently predict adverse clinical outcome. DCE-MRI delineates the importance of angiogenesis in AML and may help identify high-risk patients

for tailored anti-angiogenesis therapy, as well as monitor treatment response.

## Authorship and Disclosures

H-AH was responsible for the literature collection, data management, study design, and manuscript writing; TT-FS was responsible for coordinating the study, integrating the whole research, data management and interpretation, and manuscript writing; C-YL was responsible for the statistical analysis and interpretation of the statistical findings; B-BC calculated the dynamic MRI results and interpreted the data; J-LT, MY, S-YH and W-CC participated in the study design, patient care, and laboratory collection; C-YH collected and managed the MR raw data while H-FT planned, designed, and coordinated the study over the entire period.

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