Platelet production and related pathophysiology in acute myelogenous leukemia at first diagnosis: Prognostic implications

DIMITRIOS T.P. TRAFALIS[1], ELIAS POULAKIDAS[2], VIOLETA KAPSIMALI[3], CHRISTOS TSIGRIS[4], XENOFON PAPANICOLAOU[5], NIKOLAOS HARHALAKIS[6], EMMANUEL NIKIFORAKIS[6] and CHRISANTHI MENTZIKOF-MITSOULI[5]

[1]Department of Medical Oncology-A, Metaxa Cancer Hospital, Piraeus; [2]First Department of Internal Medicine, 401 Army Hospital; [3]Department of Immunology, 'Evangelismos' General Hospital; [4]First Surgery Clinic, 'Laikon' General Hospital, Medical School, University of Athens, Athens; [5]Department of Hematology, Metaxa Cancer Hospital, Piraeus; [6]Hematological Clinic, 'Evangelismos' General Hospital, Athens, Greece

Received October 4, 2007; Accepted November 5, 2007

Abstract. Among various laboratory and clinical features megakaryocytopoiesis and platelet (PLT) counts have been previously insufficiently evaluated for their prognostic significance in acute myelogenous leukaemia (AML). We studied several clinical and laboratory features of 108 first diagnosed AML patients in relation with their prognosis. Patients with favourable prognostic features were excluded from the study. This study focused on the prognostic impact of PLT counts and related molecular biology in AML patients at initial diagnosis. In particular, the PLT counts were correlated with the endogenous production of thrombopoietin (TPO), c-mpl expression, $CD34^+$ leukemic blast cell proportion, cytogenetics, and a prognostic correlation was established. We found that the most favorable prognosis appeared in the AML patient group with PLTs $<25x10^9$/l and correlated to cytogenetic findings (normal or abnormal karyotypes), while by far the most unfavorable prognosis was found in the patient group with PLTs $\geq 130x10^9$/l independent of the corresponding cytogenetics. It was demonstrated that AML patients with normal or elevated PLT counts at first presentation, may constitute a distinct patient group with particular characteristics such as higher levels of endogenous TPO production, high expression of $CD34^+$ leukemic blast cells, higher expression of c-mpl and consequently low response to chemotherapy and a very poor prognosis. These correlations between PLTs production (megakaryothrombopoiesis), TPO serum levels and TPO receptor (c-mpl) expression may help in the determination of risk-adapted AML patient groups and of targeted therapeutic strategies.

Correspondence to: Dr Dimitrios Trafalis, 15 Larnakos Str., Ag. Dimitrios, 17341 Athens, Greece
E-mail: dtrafalis@energonbio.com; dtrafalis@aim.com

Key words: acute myelogenous leukemia, c-mpl, megacaryocytopoiesis, platelets, prognosis, thrombopoietin, thrombopoiesis

Introduction

In acute myelogenous leukemia (AML) all myeloid lineages and differentiation pathways are affected directly or indirectly up to a certain degree. Megakaryocytopoiesis represents one of these differentiation pathways that haemopoietic stem cells may enter. In AML, megakaryocytopoiesis and thrombopoiesis defects vary, presenting as hypoplastic or absent megakaryocytopoiesis [which results in reduced numbers of platelets (PLT) or even severe thrombocytopenia, PLT: <25,000/μl], or as normal or dysplastic or hyperplastic thrombothrophic features (which result in normal or elevated, in the case of thrombocytosis, PLT numbers). Leukemia blast clonal chromosome rearrangements of 3q21 or 3q26 have been correlated with the AML cases presenting normal or elevated PLT counts (1). Although low PLT counts at the time of AML diagnosis can be interpreted as the result of suppression of normal hematopoiesis by a leukemia clone that lacks megakaryocytic differentiation, AML still represents a clonal stem cell disorder. Thus, the effectiveness of thrombopoiesis may be an important factor in disease pathophysiology and evolution (2,3).

Although several clinical and laboratory parameters, including platelet counts and megakaryocytopoiesis, have been previously studied in order to designate prognosis in AML, the prognostic value of PLT production has not been clearly determined (3,4-7).

Thrombopoietin (TPO) is a growth factor for megakaryocyte progenitor cells and can also modulate platelet function. Among the growth factor receptors that have been shown to be expressed by human AML blasts is the cloned receptor for thrombopoietin, c-mpl, which reveals considerable sequence similarity to other receptors of the class I hematopoietin receptor superfamily. Within the physiological

hematopoiesis, c-mpl is expressed on megakaryocytes, platelets, and on a large fraction of CD34 cells from which TPO enhances megakaryocyte formation and maturation (8,9). TPO levels and c-mpl expression seem to be involved in the growth of myeloid leukemia and may be related with the immaturity of the transformed progenitor cells and consequently with the prognosis of AML (10).

We studied the prognostic implications of several clinical and laboratory features of 108 first diagnosed AML patients. Patients with favourable prognostic features were excluded. The objectives of this study focused on the prognostic impact of PLT counts and related molecular biology. The PLT counts in the studied patients were correlated with the endogenous production of thrombopoietin (TPO), c-mpl expression, $CD34^+$ leukemic blast cell proportion, cytogenetics, and a prognostic correlation was established.

Materials and methods

Patient inclusion and classification. We studied first diagnosed adult AML patients (pts). Patients with good prognostic features such as with karyotypic abnormalities like paracentric inversion of chromosome 16 or translocations 8;21 and 15;17, promyelocytic leukemia (M3), and those treated with allogenic bone marrow transplantation (ABMT) were excluded from our study (~16% in total) (11). In total, 108 pts were included in our study. Laboratory and clinical data at AML initial presentation are described in Table I. As discussed below, several of these parameters were considered of prognostic value and in certain cases they were well established, as it was previously reported (4-7). The pts were classified in three groups according to their PLT counts at diagnosis. These groups were divided into subgroups according to the cytogenetic findings (Table II). In the first subgroup, we classified the pts that revealed abnormal karyotypes (AK), while in the second subgroup the pts with normal karyotypes (NK). The AML cases were diagnosed according to morphological, cytochemical and immunophenotypical criteria, and are presented according to the French-American-British (FAB) classification (12,13). Metaphases were G-banded and karyotypes were analysed according to ISSN (14). Megakaryocytopoiesis in bone marrow aspirations was studied and classified in three types according to Jinnai *et al* (2). In the first patient group there were no detectable mega-caryocytes (type I), in the second patient group normal (type II) or slightly dysplastic megakaryocytes were detected, and in the third patient group slightly or marked dysplastic megacaryocytic changes (type III) were revealed. Platelet count which expresses the megakaryopoietic status in leukaemia as well as karyotype (abnormal or normal) were evaluated either as correlated or as independent prognostic parameters. Complete response in percentage (%CR), mean duration of CR and 2-year disease-free survivors (2y-DFS) in patient groups and subgroups are also demonstrated (Table II). As first induction therapy all pts received: indarubicin 12 mg/m^2 day (d)1-d3, Ara-c 100 mg/m^2 in continuous infusion (c.i.) d1-d7. The revaluation of bone marrow on day 14 and 28 followed. Second induction therapy: indarubicin 12 mg/m^2 d1-d3, Ara-C 100 mg/m^2 c.i., dl-d5 or d7. Consolidation therapy: Ara-C 3 g mg/m^2 c.i. for 3 h/24 h for 4 days. The majority of pts received for maintenance therapy: mitoxantrone 10 mg/m^2 d1-d3, etoposide 75 mg/m^2 d1-d5.

TPO assessment. Concentrations of TPO in sera were determined using specific ELISA analysis (Quantikine ELISA assays; R&D Systems Europe, UK) as previously described (15). Analysis was performed strictly according to the manufacturer's instructions with standards and sample dilutions prepared in the supplied diluents. Standard curves were determined using the mean of duplicate analyses. TPO minimal detectable levels in serum samples were 20 pg/ml.

Determination of CD34 expression. The determination of CD34 expression on mononuclear cells that were collected with Ficoll-separation from the peripheral blood of the studied pts was performed with flow cytometry methods. Cell suspensions (50 μl) were incubated with 10 ml phycoerythrin-conjugated HPCA-2 (anti-CD34 PE; Becton Dickinson, San Jose, CA) for 20 min at 4˚C. After washing, 10,000 events were counted using a Coulter flow cytometer. The blast populations were gated using scatter parameters. An irrelevant, isotype-matched MoAb was used as negative control. Data were analyzed and CD34 expression >10% of the blast population was interpreted as significant expression (16).

c-mpl protein determination. The c-mpl protein was quantified by the solid-phase RIA method as previously described (17,18). RIA plates were coated overnight at 4˚C with 5 mg of protein extracted from peripheral blood mononuclear cells of patients and normal individuals (control) in 50 ml PBS. After washing with PBS and blocking with 100 ml 1% BSA for 1 h at 37˚C, the plates were incubated overnight at 4˚C with 50 ml mouse anti-c-mpl antibody (Genezyme, Cambridge, MA) diluted 1:500 in PBS containing 1% BSA. Then, plates were washed with PBS and amplified with rabbit anti-mouse-IgG antisera and after washing, were developed with excess iodine-125-labeled protein A [200,000 cpm (50 IU)] for 2 h at room temperature, and the contents of each well were counted with a gamma counter. The assays were performed in triplicate. The median c-mpl expression detected in the normal samples was assigned a score of 1.0, and levels in AML were expressed in proportion to this value.

Statistics. A multifactorial statistical analysis with the use of paired (Bonferroni T tests) and multiple (Duncan's multiple range test) comparisons between the examined clinical, laboratory and experimental variables among patient subgroups were performed. Chi-square test, Wilcoxon and T-test as well as the Spearman two-tailed correlation analysis were demonstrated according to statistical needs. Prognostic significance was determined. A P-value <0.05 was considered statistically significant. Only statistically significant observations are shown in the results.

Results

The clinical and laboratory features performed at initial presentation in the AML patients that were included in our study are presented in Table I.

Table I. Patient clinical and laboratory features performed at initial presentation.[a]

	Group 1 PLT: <25x10^9/l	Group 2 PLT: 25-130x10^9/l	Group 3 PLT: >130x10^9/l
Patient number (n)	32	52	24
Age (years)	48.9 (18-61)	51.2 (19-65)	50.3 (25-66)
Males/Females	18/14	28/24	13/11
FAB types	3 M0, 12 M1, 3 M2, 11 M4, 3 M6	30 M1, 15 M4, 7 M5	10 M1, 4 M2, 4 M4, 6M5
Bone marrow blast cellularity (%)	80 (40-100)	83.2 (60-100)	77.3 (50-95)
Peripheral blood blast cells (%)	66.3 (20-96)	70.5 (40-100)	53.5 (10-80)
Mean haematocrit (%)	25.4 (21-44)	25.9 (17-34)	28.5 (24-37)
Mean WBC counts (x10^9/l)	30,800	39,200	14,200
Patients with abnormal liver functional tests (LFTs) (%)	29	34	1
LDH (iU/l)	880 (120-2000)	591 (105-1640)	323 (130-1000)
Incidence of fever (>38.5˚C) (%)	62.5	58	42
Incidence of hepatomegaly (%)	15.5	24	23.1
Incidence of splenomegaly (%)	25	42	0
Incidence of hemorrhagic symptoms (%)	37.5	8	0
Incidence of microbial infection after induction therapy (%)	87.5	92	37.5

[a]Data are expressed as mean values or mean percentages. Value ranges are shown in parentheses.

Table II. Percentage of complete remission (CR), mean duration of CR and 2-year DFS in patient groups and subgroups, classified according to platelet (PLT) counts and to karyotype (normal, NK or abnormal, AK), are presented.[a]

	Percent of complete remission (CR)	CR - Mean duration (months)	2-year DFS
Group 1			
Pts with PLT: <25x10^9/l (n=32/108, 30%)	75	13.2 (<0.007)	6/32
Pts with PLT: <25x10^9/l + AK (n=22)	73	13.6	3/22
Pts with PLT: <25x10^9/l + NK (n=10)	80	12.3	3/10
Group 2			
Pts with PLT: 25-130x10^9/l (n=52/108, 48%)	65	6.8	5/52
Pts with PLT: 25-130x10^9/l + AK (n=31)	65	4.5 (<0.001)	2/31
Pts with PLT: 25-130x10^9/l + NK (n=21)	67	10.3	3/21
Group 3			
Pts with PLT: >130x10^9/l (n=24/108, 22%)	50	5.6 (<0.003)	0/24 (<0.001)
Pts with PLT: >130x10^9/l + AK (n=12)	50	5.5 (<0.004)	0/12 (<0.001)
Pts with PLT: >130x10^9/l + NK (n=12)	50	5.7 (<0.005)	0/12 (<0.005)
Pts in total (n=108)	65	8.8	10/108
Pts in total with AK (n=66, 61%)	63	8.1	5/66
Pts in total with NK (n=42, 39%)	67	9.9	5/42

[a]The values of each group and subgroup were compared with the respective values for the rest of the pts and significance levels ($p<0.05$) are shown.

Table III. Mean levels of endogenous TPO (pg/ml) and relative median c-mpl expression in blood samples of the studied patients; percent of patients that showed $CD34^+$ on leukemic blast cells; significance levels (P) in comparison to control values, are demonstrated.

	Mean TPO levels (range) (pg/ml)	P	Relative median c-mpl expression[a]	P	$CD34^+$ (%)
Normal subjects (control) (n=30)	71 (21-98)	-	1.0	-	
Patients with PLT: $<25x10^9$/l (n=32/108, 30%)	231 (50-1798)	<0.02	2.95	<0.01	40.6
Patients with PLT: 25-$130x10^9$/l (n=52/108, 48%)	184 (36-1342)	<0.05	2.72	<0.01	30.7
Patients with PLT: $>130x10^9$/l (n=24/108, 22%)	349 (107-2082)	<0.01	4.82	<0.01	100.0
Patients in total (n=108)	234 (33-2082)	<0.02	3.24	<0.01	44.6

[a]Levels are in proportion to median c-mpl levels in normal controls. Normal control median is 1.0.

The most frequent karyotypic findings were trisomy of chromosome 8 and paracentric invertion of chromosome 3 found in 10 and 6 pts, respectively. Sixty-five pts (65/108, 60%) presented chromosomal aberrations. Twelve pts of the third study group (50%) showed the following karyotypic anomalies: inv(3)(q21q26) (in 6 pts), del(7)(p12p21) (in 3 pts), t(3;3)(q21;q26) (in 2 pts), and del(1l)(q22) (in one patient). Thrombocytosis (PLTs $>500x10^9$/l) appeared in the first six pts with inv(3).

Prognostic parameters in the groups of the AML patients are shown in Table II. Our results indicate that the percentage of complete remission (CR) was independent of normal (NK) or abnormal karyotype (AK) in the three patient groups. Nevertheless, a trend to lower remission rates from pts with PLTs $<25x10^9$/l (Group 1) and NK (80% CR) to pts with PLTs $>130x10^9$/l (50% CR) (Group 3) was evident. Also, the mean duration of CR was independent of NK or AK in the total number of pts. However, in Group 2 the mean duration of CR in pts with AK was significantly lower ($p<0.001$). Moreover, it appeared to be significantly longer in Group 1 and significantly shorter in Group 3, while it was independent of the presence of AK in these two groups. Group 2 represents the majority of the pts (48%), and an intermediate mean CR duration was found. The 2-year disease-free survival (2y-DFS) was nilpotent among the 24 pts of Group 3. The presence of AK showed prognostic significance only in Group 2. Prognosis in Group 3 was severe, showing the lowest CR rates and CR duration, as well as null 2y-DFS. No correlation between PLT counts and AML subtypes in the studied patients was deduced.

The mean values of endogenous TPO levels and of the expression of its receptor (c-mpl) were significantly higher than normal subjects in all groups of patients, while the highest values were demonstrated in Group 3 patients (Table III). Moreover, a significant expression of $CD34^+$ leukemic blast population was found in 100% of patients in Group 3 (Table III).

In summary, AML patients at first diagnosis exhibit PLT counts $>130x10^9$/l, chromosome 3 abnormalities, poor prognosis, higher production of TPO, and higher expression of c-mpl with high frequency. Furthermore, a significant expression of $CD34^+$ blast cells appears in all the cases.

Discussion

Although that platelet counts under 20,000 $25x10^9$/l in AML patients are associated with a substantial risk of life-threatening hemorrhage (19), our study indicated that the best prognosis was observed in Group 1 (PLTs $<25x10^9$/l). On the other hand, the worst prognosis was found in Group 3 (PLTs $>130x10^9$/l). Group 3 pts presented the most favorable prognostic features of lower white blood cell (WBC) counts, lower percentage of peripheral blood blast cells, lower incidence of high temperature, lower LDH, higher hematocrit, no incidence of abnormal liver function, no demostration of splenomegaly, no demostration of hemorrhagic symptoms and lower incidence of infections (Table I) (4-7). Thus, the status of thrombopoiesis and the PLT production in AML seem to play an important prognostic role. The low PLT counts in AML may reflect the depression of megakaryopoiesis by the leukemic blast cell propagation, while in the case of AML with normal or elevated PLT count the megakaryopoiesis is also primarily involved with the leukemic transformation signifying hyperplastic properties. In the latter case, the malignant impairment is found in more immature stages of hemopoiesis affecting the megakaryopoietic cell lineage. This view can be supported by the observation that a significant expression of CD34 on leukemic blast population was found in 100% of patients in Group 3. A hypothesis that could interpret unfavorable prognosis in AML patients with normal or elevated PLT counts at initial diagnosis is the case of a group of secondary leukemia patients having evolved from a fast-progressed myelodysplastic syndrome (MDS).

Thrombopoietin (TPO), the ligand for c-mpl, is a glycoprotein promoting the proliferation of megakaryocytic precursors, and subsequently their differentiation into megakaryocytic and platelet cell lines (20,21). Zwierzina *et al* showed that endogenous TPO production is upregulated by a decreased circulating platelet count only in patients with refractory anemia (22). In the more advanced stages of MDS

where the leukemic clone has further progressed, an inadequate TPO response occurs, possibly due to overexpression of the mpl receptor by the malignant clone. An elevation in the endogenous levels of TPO, IL-6 and IL-8 in the thrombocytopenic patients with AML and MDS was observed by Hsu *et al* (23). We also recorded a significant elevation of endogenous TPO (Table III) in thrombocytopenic AML patients (Groups 1 and 2). Unexpectedly, the patients with normal or elevated PLT counts (Group 3) presented the highest TPO levels. In AML patients endogenous TPO may increase proportionally with PLT production and this increase is not the compensative effect of thrombocytopenia alone. Moreover, in AML patients with normal or elevated PLT counts, stem cell disorders involving TPO overproduction or c-mpl dysfunctional mutations may occur. Furthermore, as TPO overexpression is detected in several AML cases, it promotes the proliferation of leukemic blasts (24). The possibility of an increased sensitivity of megakaryoblasts to TPO has not been investigated in detail.

Despite the fact that the main clinical importance of chromosome aberrations in hematological disorders is diagnostic, in several AML cases it appears to be of prognostic value. Abnormalities of 3q chromosome are considered of poor prognosis in AML (11,25). However, in our report no differences occurred in prognostic parameters between the patients with or without 3q chromosome aberrations in the third study group. Although rearrangements of chromosome 3q in AML are strongly associated with normal or elevated PLT counts, other random chromosome alterations or normal karyotypes have also been reported in such cases (1,3).

C-mpl proto-oncogene encodes a member of the cytokine receptor superfamily. We have studied the expression of c-mpl in a series of 105 patients with hematologic malignancies using Northern blot analysis. While the levels of c-mpl transcripts in lymphoid malignancies and in chronic myeloproliferative disorders are not significantly different from those found in normal bone marrow cells, the c-mpl expression is increased in patients with AML and in MDS. There is no significant correlation between c-mpl expression and the FAB classification of AML. Results from several studies suggest that c-mpl protein overexpression in AML plays a role in the aggressiveness of disease and is of prognostic relevance. Patients with high c-mpl expression appeared to belong to a subgroup of AML with a low rate of complete remission and a poor prognosis, including secondary leukemia and AML with unfavourable cytogenetic abnormalities (10,16,17,26). However, no overexpression of c-mpl protein or mRNA was found in typical 3q syndrome AML cases (27). Moreover, in our study the c-mpl levels were directly correlated with the prognosis in the three AML patient groups and the highest c-mpl expression was observed in AML patients with normal or elevated PLT counts (Group 3, Table III). Chelvatheebam *et al* summarized the incidence and prognostic significance of c-mpl expression in AML (28), and more recently Corrazza *et al* suggested that in patients with AML, TPO levels could be secondary to TPO clearing by functional c-mpl receptor myeloid blast cells and that TPO may serve as an *in vivo* myeloid leukemic growth factor in a significant number of patients (29).

Conclusively, AML patients with normal or elevated PLT counts may constitute a distinct patient group with particular characteristics such as high levels of endogenous TPO production, high expression of $CD34^+$ leukemic blast cells, higher expression of c-mpl and consequently low response to chemotherapy and very poor prognosis. Our data suggest a need for further studies in an attempt to develop risk-adapted AML targeted therapies and treatment strategies.

References

1. Grigg AP, Gascoyne RD, Phillips GL and Horsman DE: Clinical, haematological and cytogenetic features in 24 patiens with structural rearrangements of the Q arm of chromosome 3. Br J Haematol 83: 158-165, 1993.
2. Jinnai I, Tomonaga M, Kuriyama K, Matsuo T, Nonaka H, Amenomori T, Yoshida Y, Kusano M, Tagawa M and Ichimaru M: Dysmegakaryocytopoiesis in acute leukaemias: its predominance in myelomonocytic (M4) leukaemia and implication for poor response to chemotherapy. Br J Haematol 66: 467-472, 1987.
3. Hoyle C and Hayhoe FG: Abnormal megakaryopoiesis in acute leukaemia. Br J Haematol 68: 393-394, 1988.
4. Brandman J, Bukowski RM, Greenstreet R, Hewlett SJ and Hoffman CG: Prognostic factors affecting remission, remission duration, and survival in adult acute non-lymphocytic leukemia. Cancer 44: 1062-1065, 1979.
5. Keating JM, Smith TL, Gehan EA, McCredie KB, Bodey GP and Freireich JM: Prognostic factor analysis for use in development of predictive models for response in adult acute leukemia. Cancer 50: 457-465, 1982.
6. Passe S, Mike V, Mertelsmann R, Gee ST and Clarkson DB: Acute non-lymphoblastic leukemia. Prognostic factors in adults with long-term follow-up. Cancer 50: 1462-1471, 1982.
7. Smith TL, Gehan EA, Keating JM and Freireich JM: Prediction of remission in adult acute leukemia. Development and testing of predictive models. Cancer 50: 466-472, 1982.
8. Vigon I, Mornon JP, Cocault L, Mitjavila MT, Tambourin P, Gisselbrecht S and Souyri M: Molecular cloning and characterization of h-mpl, the human homolog of the v-mpl oncogene: Identification of a new member of the hematopoietic growth factor receptor superfamily. Proc Natl Acad Sci USA 898: 5640-5644, 1992.
9. Zeigler FC, de Sauvage F, Widmer HR, Keller GA, Donahue C, Schreiber RD, Malloy B, Hass P, Eaton D and Matthews W: *In vitro* megakaryocytopoietic and thrombopoietic activity of c-mpl ligand (TPO) on purified murine hematopoietic stem cells. Blood 84: 4045-4052, 1994.
10. Wetzler M, Baer MR, Bernstein SH, Blumenson L, Stewart C, Barcos M, Mrozek K, Block AW, Herzig GP and Bloomfield CD: Expression of c-mpl mRNA, the receptor for thrombopoietin, in acute myeloid leukemia blasts identifies a group of patients with poor response to intensive chemotherapy. J Clin Oncol 15: 2262-2268, 1997.
11. Arthur DC, Berger R, Golomb HM, Swansbury GJ, Reeves BR, Alimena G, Van Den Berghe H, Bloomfield CD, de-la-Chapelle A and Dewald GW: The clinical significance of karyotype in acute myelogenous leukemia. Cancer Genet Cytogenet 40: 203-216, 1989.
12. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR and Sultan G: Proposed revised criteria for the classification of acute myeloid leukaemia: a report of the French-American-British Cooperative Group. Ann Med Interne 103: 620-624, 1985.
13. Clark EA and Lanier LL: Report from Vienna: in search of all surface molecules expressed on human leukocytes. J Clin Immunol 9: 265-272, 1989.
14. ISCN: An international system for human cytogenetic nomenclature. Cytogenetics and Cell Genetics. Mitelman F (ed). Karger, Basel, 1991.
15. Foss B, Nesthus I, Bergheim J and Bruserud O: Serum levels of thrombopoietin and stem cell factor in acute leukemia patients with chemotherapy-induced cytopenia and complicating infections. Platelets 10: 17-23, 1999.
16. Schroder JK, Kolkenbrock S, Tins J, Kasimir-Bauer S, Seeber S and Schutte J: Analysis of thrombopoietin receptor (c-mpl) mRNA expression in *de novo* acute myeloid leukemia. Leuk Res 24: 401-409, 2000.

17. Albitar M, Manshouri T, Kantarjian H, Keating M, Estrov Z, Faber J, Freireich EJ, Pierce S and Estey E: Correlation between lower c-mpl protein expression and favorable cytogenetic groups in acute myeloid leukemia. Leuk Res 23: 63-69, 1999.
18. Kaban K, Kantarjian H, Talpaz M, O'Brien S, Cortes J, Giles FJ, Pierce S and Albitar M: Expression of thrombopoietin and its receptor (c-mpl) in chronic myelogenous leukemia. Correlation with disease progression and response to therapy. Cancer 88: 570-576, 2000.
19. Gaydos LA, Freireich EJ and Mantel N: The quantitative relation between platelet count and and hemorrhage in pts with acute leukemia. N Engl J Med 266: 905-909, 1962.
20. De Sauvage FJ, Hass PE, Spencer SD, Malloy BE, Gurney AL, Spencer SA, Darbonne WC, Henzel WJ, Wong SC and Kuang WJ: Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature 369: 533-538, 1994.
21. Wendling F, Maraskovsky E, Debili N, Florindo C, Teepe M, Titeux M, Methia N, Breton-Gorius J, Cosman D and Vainchenker W: c-MPL ligand is a humoral regulator of megakaryocytopoiesis. Nature 369: 571-574, 1994.
22. Zwierzina H, Rollinger-Holzinger I, Nuessler V, Herold M and Meng YG: Endogenous serum thrombopoietin concentrations in patients with myelodysplastic syndromes. Leukemia 12: 59-64, 1998.
23. Hsu HC, Lee YM, Tsai WH, Jiang ML, Ho CH, Ho CK and Wang SY: Circulating levels of thrombopoietic and inflammatory cytokines in patients with acute myeloblastic leukemia and myelodysplastic syndrome. Oncology 63: 64-69, 2002.
24. Matsumura I, Kanakura Y, Ikeda H, Ishikawa J, Yoshida H, Horikawa Y, Nishiura T, Tahara T, Kato T, Miyazaki H and Matsuzawa Y: Coexpression of thrombopoietin and c-mpl genes in human acute myeloblastic leukemia cells. Leukemia 10: 91-94, 1996.
25. Horsman DE, Gascoyne RD and Barnett MJ: Acute leukemia with structural rearrangements of chromosome 3. Leuk Lymphoma 16: 369-377, 1995.
26. Vigon I, Dreyfus F, Melle J, Viguié F, Ribrag V, Cocault L, Souyri M and Gisselbrecht S: Expression of the c-mpl proto-oncogene in human hematologic malignancies. Blood 82: 877-883, 1993.
27. Bouscary D, Fontenay-Roupie M, Chretien S, Hardy AC, Viguie F, Picard F, Melle J and Dreyfus F: Thrombopoietin is not responsible for the thrombocytosis observed in patients with acute myeloid leukemias and the 3q21q26 syndrome. Br J Haematol 91: 425-427, 1995.
28. Chelvatheebam S, Langabeer SE, Linch DC, Hills RK and Greenwell P: Incidence and prognostic significance of C-MPL expression in acute myeloid leukemia. Leuk Res 27: 869-870, 2003.
29. Corazza F, Hermans C, D'Hondt S, Ferster A, Kentos A, Benoit Y and Sariban E: Circulating thrombopoietin as an *in vivo* growth factor for blast cells in acute myeloid leukemia. Blood 107: 2525-2530, 2006.