Multiparametric approach of prognostic factors in acute myeloid leukemia – a bicentric study

Abordarea multiparametrică a factorilor de prognostic în leucemia acută mieloidă – studiu bicentric

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Abstract

Acute myeloid leukemias (AML) are a group of malignant hematologic disorders with varying clinical, morphologic, immunologic and molecular characteristics. Prognostic factors evaluation remains an important subject of study in order to improve the outcome of patients with AML, a disease with poor prognostic by itself. Establishing prognosis at the time of diagnosis is expected in order to stratify treatment. The aim of this study is to evaluate some prognostic factors in AML: age, leukocyte count, platelet count, FAB (French-American-British Cooperative Group) subtype, LDH serum level, immunophenotype at diagnosis. We included 59 patients with primary or secondary AML at the time of diagnostic, with complete investigation at the Hematology Department of Medical Clinic I in Tg-Mureş and at the Hematology Department of "Ion Chiricuță" Cancer Institute Cluj-Napoca. Our results indicate that leukocyte count over 100000/µL, LDH serum activities over 1000 U/L and C34+CD56+ association are significant prognostic factors in AML at the time of diagnostic. FAB subtypes M0, M1, M4 significantly influenced complete remission. Due to the low number of cases prognostic evaluation of cytogenetics and molecular findings is not possible. These results represent an intermediary evaluation of patients, because the study is still underway.

Keywords: *AML, prognostic, immunophenotyping*

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Rezumat

Leucemiile acute mieloide (AML) reprezintă un grup de afecţiuni hematologice maligne cu caracteristici clinice, morfologice, imunologice şi moleculare variate. Evaluarea factorilor prognostici rămâne o importantă temă de cercetare pentru a îmbunătăţi evoluţia pacienţilor cu LAM, boală cu prognostic nefavorabil ca atare. Stabilirea prognosticului la diagnostic este de dorit pentru a putea stratifica tratamentul. Scopul studiului este evaluarea la diagnostic a unor factori prognostici în LAM : vârsta, numărul de leucocite, numărul de trombocite, subtipul FAB, activitatea serică al LDH, imunofenotipul. În studiu am inclus 59 pacienţi cu LAM primară sau secundară, investigaţi în Departamentul de Hematologie al Clinicii Medicale I din Tg. Mureş şi în Departamentul de Hematologie al Instutului Oncologic "Ion Chiricuţă" din Cluj Napoca. Rezultatele obţinute arată că la diagnostic : numărul de leucocite peste 100000/µL, activitatea serică a LDH peste 1000 U/L, asocierea CD34+CD56+, sunt factori de prognostic în LAM. Subtipurile FAB M0, M1, M4 au influenţat semnificativ obţinerea remisiei complete. Datorită numărului mic de cazuri evaluarea prognostică a citogeneticii şi testelor de biologie moleculară nu este deocamdată posibilă. Aceste rezultate reprezintă o evaluare intermediară, deoarece studiul este încă în derulare.

Cuvinte-cheie: *LAM, prognostic, imunofenotipare*

Introduction

Acute myeloid leukemias (AML) are a group of malignant hematologic disorders with varying clinical, morphologic, immunologic and molecular characteristics. It is known that cytogenetics is the most important prognostic factor in AML (1). Flow cytometric immunophenotyping is an indispensable technique that allows differentiation between normal cells and leukemic cells, definition of maturation stage and aberrant phenotypes' recognition, and its use is necessary for the diagnosis, monitoring and prognosis of leukemias. Establishing prognosis at the time of diagnosis is expected in order to stratify treatment.

Aim of study

We propose a longitudinal survival study, with 3 years follow-up, aimed to evaluate some prognostic factors in AML: age, leukocyte count, platelet count, FAB (French-American-British Cooperative Group) subtype, lactate dehydrogenase (LDH) serum activity, immunophenotype and cytogenetics at diagnosis. The study included 59 patients with AML.

Materials and methods

Fifty nine adult patients with newly diagnosed AML were included in the study at the Hematology Department of Medical Clinic I in Tg-Mureş and at the Hematology Department of "Ion Chiricuţă" Cancer Institute Cluj-Napoca, between January 2006 and December 2008.

The inclusion criterion was: untreated patients with primary or secondary AML at the time of diagnostic, with complete investigation.

The investigation protocol was structured as follows:

- Biological products sampling: peripheral blood (PB) and bone marrow (BM);
- Hematology routine investigations – complete blood count: white blood cells, platelet count, hemoglobin, haematocrit;
- Biochemistry routine investigations
- Usual staining: May Grunwald Giemsa (MGG) for PB and BM smears;
- Microscopic morphologic examination;
- Cytochimic staining for peroxidase and periodic acid Schiff (PAS);
- Immunophenotyping analysis of PB or BM samples, using monoclonal antibodies by flow cytometry. Immunophenotyping analysis was performed in Hematology Department of Emergency Clinical Hospital Mureş (Becton Dickinson FacsScan) and Flow-cytometry Laboratory of Ulm University, Germany.

The immunophenotype was determined using erythrocyte lysed and wash protocol of

whole ethylenediamine tetracetic acid (EDTA) PB or BM (as case) samples that were obtained at diagnosis.

We used a large panel of 3 color combination of monoclonal antibodies: fluorescein isothiocyanate, phycoerythrin, peridinin-chlorophyll-protein complex (FITC; PE; PerCP):

- CD34;CD13;CD45
- CD34;CD33;CD45
- CD34;CD117;CD45
- HLA-DR;CD34;CD45
- CD56;CD34;CD45
- CD64;CD11b;CD45
- CD14;CD36;CD45
- CD19;CD34;CD45
- CD10;CD34;CD45
- CD19;CD34;CD45
- CD7;CD34;CD45

Blasts selection was made using CD45 (dim expression) and sideward scatter SSC (low expression). Surface antigens were considered positive when 20% of cells or more were positive compared with isotype control.

- Cytogenetic and Molecular analysis - Laboratory of Cytogenetic and Molecular Diagnostic of Ulm University, Germany. Karyotyping was applied in 33 patients and in those cases with normal karyotype the mutational status of the nucleophosmin (NPM1) and related tyrosine kinase 3 (FLT3) genes was assessed. This analysis was possible thanks to collaboration between "Ion Chiricuță" Cancer Institute Cluj-Napoca and the University of Ulm, Germany.

Based on cytogenetics these patients were divided in three prognostic groups:

- Good prognostic group: t(8;21), t(15;17), inv16
- Intermediate prognostic group: normal karyotype, +21, +22,+ 8, +11, 7q-,9q-
- Poor prognostic group: complex karyotype, inv 3, 5-, 7-, 5q-,

When combining cytogenetics with molecular analysis we divided our patients in two groups:

- Favorable profile – good or intermediate prognostic group and NPM1+
- Unfavorable profile – poor or intermediate prognostic group and FLT3+, with or without NPM1

Overall survival (OS) was calculated from the date of registration until death or last follow-up. Complete hematologic remission (CR) was defined according to standard criteria: less than 5% blast cells in BM with maturation of cell lines and restoration of peripheral blood counts. Statistical Analysis of data was done using EpiInfo and GraphPrism 4. Statistic significance between means and medians (depending upon the case – normality test Kolmogorov-Smirnov) was calculated using Student t test or Mann-Whitney test.

Survival durations were estimated with Kaplan-Meier curves and a log-rank test was used for establishing survival differences (due to studied factors).

Results

Our results represent an intermediary evaluation of patients, because the study is still underway. We analyzed 59 adult patients included in this study according to inclusion criteria at the time of analysis.

General characteristics of patients. Our group of study includes 23 women and 36 men, 30 from urban areas and 29 from rural areas. Mean age at diagnosis was 53 years. From the 59 patients, 37 were aged under 60 and 22 were aged 60 or over. 36 of our patients died during the study. The mean value of the survival period was 7 months, with a minimum of one day and a maximum of 26 months.

Distribution of patients according to FAB is provided in *Table 1*. In our group we can observe the predominance of M2 FAB subtype, followed by M4 and M1 FAB subtypes.

Analyzing the influence of FAB subtype on survival and CR we could observe that FAB subtypes M0, M1, M4, characterized by frequent expression of CD34, although they did

Table 1. Patients distribution according to FAB subtype

FAB	Frequency	Percent
M0	3	5,1%
M1	13	22,0%
M2	22	37,3%
M3	1	1,7%
M4	13	22,0%
M4Eo	1	1,7%
M5	3	5,1%
M6	2	3,4%
M7	1	1,7%
Total	59	100,0%

not significantly influence OS (p = 0.07) however OS tended to be shorter in patients with these subtypes. M0, M1 and M4 subtypes significantly influenced achieving CR (p = 0.03).

Age at diagnosis is a very well known prognostic factor in AML. Our group was divided in two subgroups: patients aged 60 or over and patients aged under 60. Difference in survival between these groups was not statistically significant (p = 0.15), but analysis of survival starting from the 10th month of disease (21 patients) confirms age as prognostic factor (p = 0.045) (*Figure 1*).

Complete blood count, routine investigation, gives us important prognostic data. White blood cells count of over 100000/µL at diagnostic had negative prognostic value on survival (p = 0.03) (*Figure 2*). The threshold value of 30000 leukocytes/µL recommended in some studies was not significant in this study.

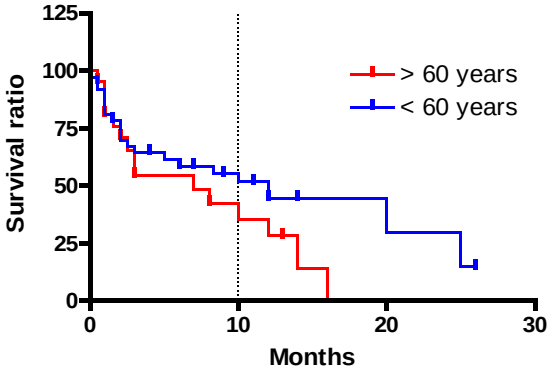

Figure 1. Difference in overall survival between patients aged 60 or over and patients aged under 60 (starting from the 10th month of disease, p = 0.045)

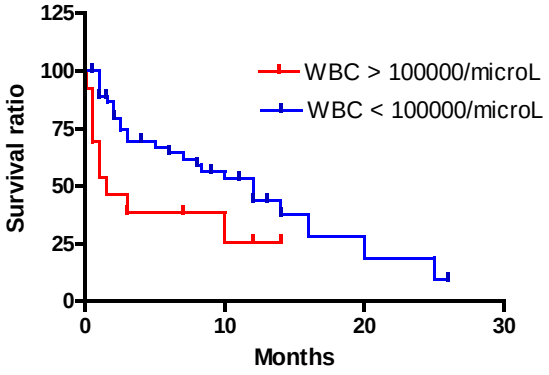

Figure 2. Overall survival and white blood cells count WBC (p = 0.03)

Platelet count < 30000/ µL at diagnostic did not influence OS in our study (p = 0.66).

Serum LDH activity - routine investigation in AML - it is also known as a prognostic factor. LDH serum activities > 1000 U/L at diagnostic had significant negative prognostic value for survival (p = 0.01) (*Figure 3*).

Analysis of leukemic cell phenotype has an important contribution in diagnosis and also for prognosis. CD34 expression was present in 76% of cases (45 patients), CD13 in

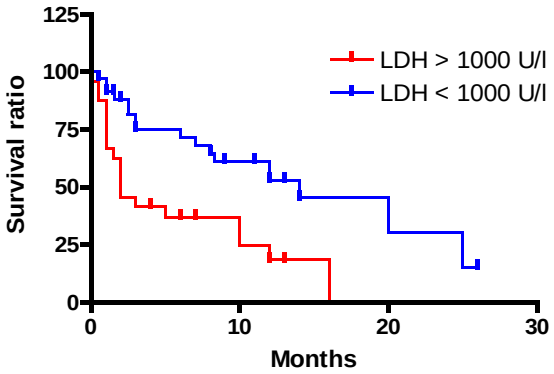

Figure 3. Overall survival and LDH serum activities (p = 0.001)

Table 2. Results of cytogenetic and molecular analysis

Karyotype	Number of patients	Molecular biology	Number of patients
Normal	13	FLT3-ITD	3
Complex	11	NPM1	3
t(8;21)	2	FLT3+NPM1	1
+22	2		
t(15;17)	1		
Inv16	1		
Inv3	1		
-5	1		
-7	1		

86% of cases (51 patients), and CD33 in 84% of cases (50 patients). The analysis panel also included lymphoid markers: CD19 was expressed in 9 cases (15%), CD7 in 7 cases (12%) and CD56 in 12 cases (20%).

We compared survival of patients with CD34+ against CD34- patients at the time of diagnostic. Although the mean of survival time in CD34+ patients was 6 month and in CD34- patients was 8 months, CD34 did not significantly correlate with OS (p = 0,14). Nevertheless, at 6 months after diagnosis CD34 had a significant value for survival (p = 0.01) (*Figure 4*).

CD34 had no influence in attaining CR after multidrug induction therapy. We studied

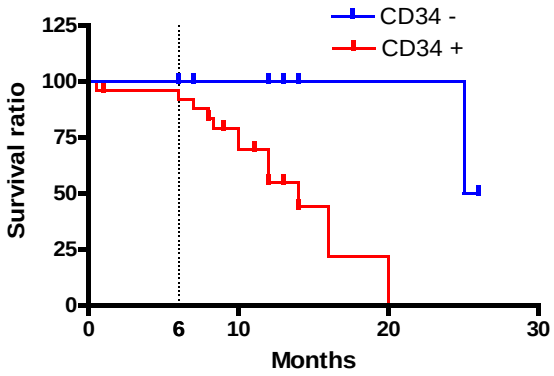

Figure 4. Overall survival and CD34 expression, at 6 months from diagnosis

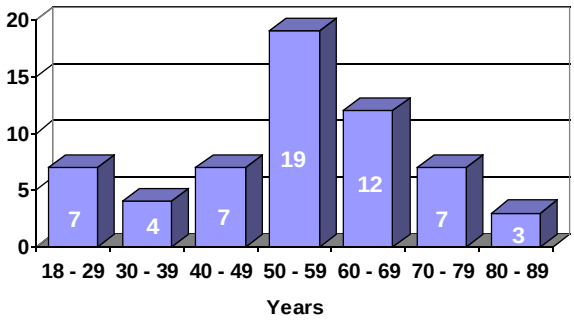

Figure 5. Age distribution of patients at diagnosis

combinations of CD markers and the way they influence patients' period of survival: CD34+/CD7+, CD34+/CD33+ respectively CD34+/CD56+ against CD34+/CD7-, CD34+/CD33- respectively CD34+/CD56-. From this combinations only CD34+CD56+ significantly influenced survival of patients (p = 0.04).

Results of cytogenetic and molecular analysis are provided in *Table 2*.

According with these results 4 of the 33 patients were included into the favorable group, 15 into the intermediate group and 14 into the poor cytogenetic prognostic group. When combining cytogenetics and molecular results 13 patients were considered with favorable profile and 20 patients with unfavorable profile. Among 28 patients treated with curative intent, 11 achieved CR: 3 of good risk group, 7 of intermediate risk group and one of poor risk cytogenetics group. Due to the small number of cases statistical analysis was not made.

Discussions

Acute myeloid leukemia (AML) may affect all age groups, however the incidence increases significantly with age, the majority of patients being diagnosed worldwide in their 6th and 7th decade of life. Suboptimal outcomes are the result of adverse biologic characteristics of leukemia in the elderly, as well as the presence of medical co-morbidities. Age distribution at diagnostic in our patients was Gaussian

and the age varied from 18 to 81 years old. We distinguished the age segment 50-59, which yielded a high frequency of disease (*Figure 5*).

Some data indicates that AML is developing from hematopoetic precursors in early stage of maturation and thus can implicate more than one hematopoetic cell line. This could explain the clinical-biological behavior of disease and prolonged neutropenia after chemotherapy. Moreover, a large number of blasts express multidrug resistance glycoprotein - MDR1, and incidence of unfavorable karyotypes is high (7-, 5-). These factors, rather than age per se, are responsible for unfavorable evolution of disease. Against AML in younger age, in elderly AML often evolves from a precedent hematologic disorder or after treatment for another malignancy. Morphologic signs of uni- or multilineage dysplasia are often seen (2, 3).

Most studies consider age over 60 as poor prognostic factor. Explanation for our findings – age is significant just after 10 month from the diagnosis, could be addressability toward medical service in advanced stages of biologic degradation, but also acute toxicity of chemotherapy that is important in patients with chronic cardiac, hepatic or renal disease. Older patients may also have lesser bone marrow regenerative capacity, even after successful leukemia cytoreduction. Their inability to tolerate long periods of pancytopenia and malnutrition are the major barriers to successful treatment (4, 5).

High white cell count at diagnosis is unanimously accepted as a poor prognostic factor (6) and we obtained the same result after statistical analysis. It is important to mention that 15 patients presented leucopenia at diagnosis.

Our study indicates that LDH serum activity is a poor prognostic factor. In other studies LDH value is a prognostic factor for OS and for disease free survival in univariate analysis and also in multivariate analysis. As it is an accessible parameter it can be used as a prognostic factor for this disease (7).

The role of immunophenotyping is clear in diagnosis of AML. Analysis panels used by different laboratories may vary. Most data indicate the necessity to introduce not only diagnostic markers, but also prognostic markers with implication for patients' outcome and treatment management in the analysis panel. The study of markers association becomes important.

CD34 antigen expression is not unanimous accepted as having a prognostic impact on OS and thus a prognostic value. Some authors consider that a high expression of CD34, frequent in AML subtypes M_0, M_1, M_4, correlates with a low rate of CR, different from those non expressing CD34 (2, 8, 9). From the point of view of other authors CD34 should not be considered a marker of poor prognosis in AML (10, 11, 12).

Co-stimulatory molecules such as lymphocyte function-associated antigen are important regulatory elements in immunological cascades, but their role in AML has been rarely investigated. A recent study demonstrated that patients with more than 8 % CD56 (neuronal cell adhesion molecule-NCAM) positive cells in the BM relapsed significantly sooner (13). In another study CD56 expression was associated with a significantly shorter overall survival, but did not affect remission rate (9). Our results indicate that CD34/CD56 association is a poor prognostic factor.

Most studies indicate that cytogenetics is the most important prognostic factor in AML (14, 15). It is known that patients with normal karyotype - AML represent the larger group and they are considered with intermediate prognostic. It is clear now that this group is very heterogeneous at the molecular level. Since 2 or more genetic alterations are present simultaneously in many patients, it is important to devise a prioritized schema that stratifies patients to risk-adapted therapies using information on all known prognostic markers (16). Some studies showed

that presence or absence of some gene mutation or alteration in gene expression could affect prognostic of this patients. So far, the most important prognostic factor in patients with normal karyotype is internal tandem duplication of FLT3 gene (ITD-FLT3).

Due to the low number of cases, in this intermediate stage of our study, prognostic evaluation of cytogenetics and molecular findings is not yet possible.

Conclusions

Our preliminary data indicate that leukocyte count over 100000/µL, LDH serum activities over 1000 U/L and C34 and CD56 association are significant negative prognostic factors in AML at the time of diagnostic. FAB subtypes M0, M1, M4 significantly influenced CR achieving.

Prognostic factors evaluation remains an important subject of study in order to improve the outcome of patients with AML, a disease with poor prognostic by itself.

Abbreviations list

AML – acute myeloid leukemia
BM – bone marrow
CR – complete remission
EDTA - ethylenediamine tetracetic acid
FAB - French-American-British Cooperative Group
FITC - fluorescein isothiocyanate
FLT3 - related tyrosine kinase 3
FLT3-ITD - internal tandem duplication of FLT3
LDH - lactate dehydrogenase
MGG - May Grunwald Giemsa
NCAM - neuronal cell adhesion molecule
NPM1 - nucleophosmin
OS – overall survival
PB – peripheral blood
PE - phycoerythrin
PerCP - peridinin-chlorophyll-protein complex
SSC - sideward scatter
WBC - white blood cells

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