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The immunophenotype of adults with acute myeloid leukemia: proposal of prognostic value

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Background. In acute myeloid leukemia (AML) patients a variety of clinical and biological parameters, including immunophenotype, have been examined for a potential value in predicting treatment response and survival. Several reports suggested a relationship between some antigens and acute myeloid leukemia prognosis, but subsequent studies produced conflicting results. With regard to these data, we attempted to evaluated the prognostic signifcance of different immunophenotyping subgroups. We also compared the results of other clinical and biological variables within the context of clinical practive of adult AML patients treated with chemotherapy.

Materials and methods. The prognostic significance of combined CD7/CD34 immunophenotype was evaluated in 42 adult acute mycloid leukemia patients using a uniform panel of monoclonal antibodies. Extensive immunophenotyping of myeloid blasts was performed using the flow cytometry method.

Results. Patients with the positive combined CD7/CD34 immunophenotype had a significantly lower complete remission rate (P = 0.01), disease-free survival (P = 0.01) and overall survival (P = 0.02). The other factors influencing achievement of complete remission, 1-year disease-free survival and 1-year overall survival were age and performance status.

Conclusions. The expression of CD7/CD34 phenotype is an independent prognostic factor in patients with adult AML. We have included in this prognostic value the CD7/CD34-positive phenotype. This value permitted a stratification of patients with acute myeloid leukemia, thereby allowing for the consideration of innovative therapies to improve outcome in the poorer outcome groups.

Key words: acute myeloid leukemia, immunophenotype, CD7/CD34 phenotype, prognostic value

INTRODUCTION

Immunophenotyping is a widely used method to diagnose and classify acute leukemias, thereby complementing morphology and cytochemistry (1–7). A variety of clinical and biological parameters, including immunophenotype, have been examined for potential value in predicting treatment response and survival (8). Several reports suggested a relationship between some antigens (*e.g.*, CD7, CD9, CD13, CD14, CD15, CD33, CD34, CD56), also P-glycoprotein (Pgp) expression and acute myeloid leukemia (AML) prognosis, but subsequent studies produced conflicting results (8–12).

Leukemic myeloblasts express a variety of leukocyte differentiation antigens, which reflect commitment to the myeloid lineage as well as the level of maturation.

With regard to these data, we attempted to evaluate the prognostic significance of different immunophenotyping subgroups. We also compared the results of other clinical and biological variables within the context of clinical practice of adult AML patients treated with chemotherapy.

Blast cells from 42 AML patients were analyzed with a uniform panel of monoclonal antibodies (mAbs).

PATIENTS, MATERIALS AND METHODS

Patients

From January 1999 through January 2002, 42 untreated AML patients (without acute promyelocytic leukemia) diagnosed in a single center were enrolled in the study. In all patients bone marrow investigation revealed a typical morphology and cytochemistry.

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These 42 patients were analyzed for the myeloid immunophenotype as defined immunologically by the expression of the following panmyeloid markers: myeloperoxidase (MPO), CD13, CD33. Acute promyelocytic leukemia patients were not analyzed in this study, because they had received retinoic acid treatment. For each patient, we analyzed several clinical and biological characteristics: age, white blood cell (WBC) count at diagnosis, performance status (WHO PS), French–American–British (FAB) morphology, lactate hydrogenase (LDH) level. Also, permeability glycoprotein (Pgp) expression was analyzed.

In order to evaluate the prognostic significance of different immunophenotyping subgroups, the patients were divided into two subgroups: CD7/CD34-positive (n = 22) and CD7/CD34-negative (n = 20). Their clinical and biological features are summarized in Table 1.

Table 1. **Patient population**

	All patients (N = 42)	CD7/CD34 pos. (n = 22)	CD7/CD34 neg. (n = 20)	P
Age, y, mean	48 (17–72)	49	47	NS
WHO PS < 2, n	26	14	12	NS
WBC at diagnosis × 10^9 per l, mean	34	30	38	NS
LDH, U/l, mean	1373	1463	1283	NS
FAB morphology, n				
M0	1	0	1	NS
M1	12	7	5	NS
M2	16	9	7	NS
M4	7	4	3	NS
M4E	1	0	1	
M5	4	2	2	NS
M6	1	0	1	NS
M7	0	0	0	NS
Pgp expression, mean, %	32	38	26	NS

The P value indicates a comparison of a patient group with the CD7/CD34-positive phenotype and a group with the absence of CD7/CD34 phenotype. The normal value of LDH is less than 420 U/l. NS – not significant.

Treatment

None of the patients had a history of prior therapies with anticancer drugs or a diagnosis of myelodisplastic syndrome. All patients in this study were given a combination of cytosine arabinoside (AraC) and anthracyclin. Antileukemic treatments were differentiated according to age, but the treatments were similar.

Patients aged 59 years or younger received 100 mg/m^2 AraC per day for 7 days and 45 mg/m^2 daunorubicin per day for 3 days. The patients who achieved complete remission (CR) received consolidation therapy with a high dose of AraC (3 g/m^2), 12 doses either of HAM regimen (high dose of AraC – 3 g/m^2, 8 doses with mitoxantrone 12 mg/m^2 per day for 3 days). Four patients received autologous hematopoietic stem cell transplantation as part of consolidation treatment. Two patients received allogeneic stem cell transplantation from matched related donors.

Patients aged 60 years and older were given the same induction treatment combination:

100 mg/m^2 AraC per day for 7 days and 45 mg/m^2 daunorubicin per day for 3 days.

The patients that achieved complete remission received consolidation therapy:

AraC 200 mg/m^2 every 12 hours for 10 days plus thioguanine 100 mg/m^2 /d orally for 10 days. Consolidation therapy cycles were repeated every 3 months to a maximum of 8 cycles (2 years of treatment).

The distribution of variables analyzed as the CD7/CD34 phenotype, WHO performance status, FAB subtypes, WBC count at diagnosis, LDH level, Pgp expression were not different between different treatment regimens. In addition, there were no significant differences in the treatment according to antigen expression.

Immunological phenotyping

Venous blood samples were collected into Vacutainer K_3 EDTA tubes (BD, UK). Further morphological blood film sample evaluation was performed using azure–eozin staining techniques and a Nikon light microscope. Automated hematology analysis was performed on automated hematology analyzers (Beckman Coulter and Abbott, USA).

AML diagnosis was confirmed by extensive immunophenotyping of myeloid blasts with a FACSCalibur™ flow cytometer (BD, USA). We used the following 14 monoclonal antibodies: CD2, CD4, CD5, CD7, CD10, CD13, CD14, CD19, CD33, CD34, CD56 HLA-DR, Pgp and MPO (BD, Pharmingen) to stain the sam-

ples. All antibodies were used for cell surface staining except MPO, which was used for intracellular staining (5–7).

Data collection and analysis was performed with CellQuest™ software, instrument set up and calibration with FACSComp™ software, Calibrite™ beads, unstained blood samples, fluorescence control samples, external quality control procedures were kindly provided by BD Heidelberg team's QC programme CEQUAL.

Membrane and cytoplasmic markers were considered positive when more than 20% of the blast cells expressed it. These values were selected by reference to the standards of the European Group for the Immunological Classification of Leukemias (4).

Statistical analysis

Clinical and biological factors were investigated for their influence on remission rate by the Fisher exact test for categorical variables and by the Mann–Whitney U test for continuous variables. Disease-free survival (DFS) was measured from ascertaining complete remission (CR) until relapse or death from any cause, with observation censored for patients last known alive without report of relapse. Overall survival (OS) was measured from diagnosis until death from any cause, with observation censored for patients last known alive. DFS and OS were estimated by the Kaplan–Meier method (13) and compared by the log-rank test. Significance was defined as $P \leq 0.05$.

RESULTS

Immunophenotype

The expression of 11 CD antigens, MPO, HLA-DR markers, Pgp and combined CD7/CD34 immunophenotype for the group of 42 adult AML patients is presented in Table 2.

Among different markers, most positive were the following myeloid lineage antigens (percentage of positivity noted in parentheses): CD13 (95%), CD33 (91%), MPO (73%).

The hematopoietic progenitor cell markers HLA-DR and CD34 were positive in 89% and 68%, respectively. CD7, a stem cell marker, was positive in 57% of patients. We detected T-cell markers CD2 in 18% of patients, CD5 in 4%, whereas B-cell markers CD19 in 16%, CD10 in 10%. CD14 was posi-

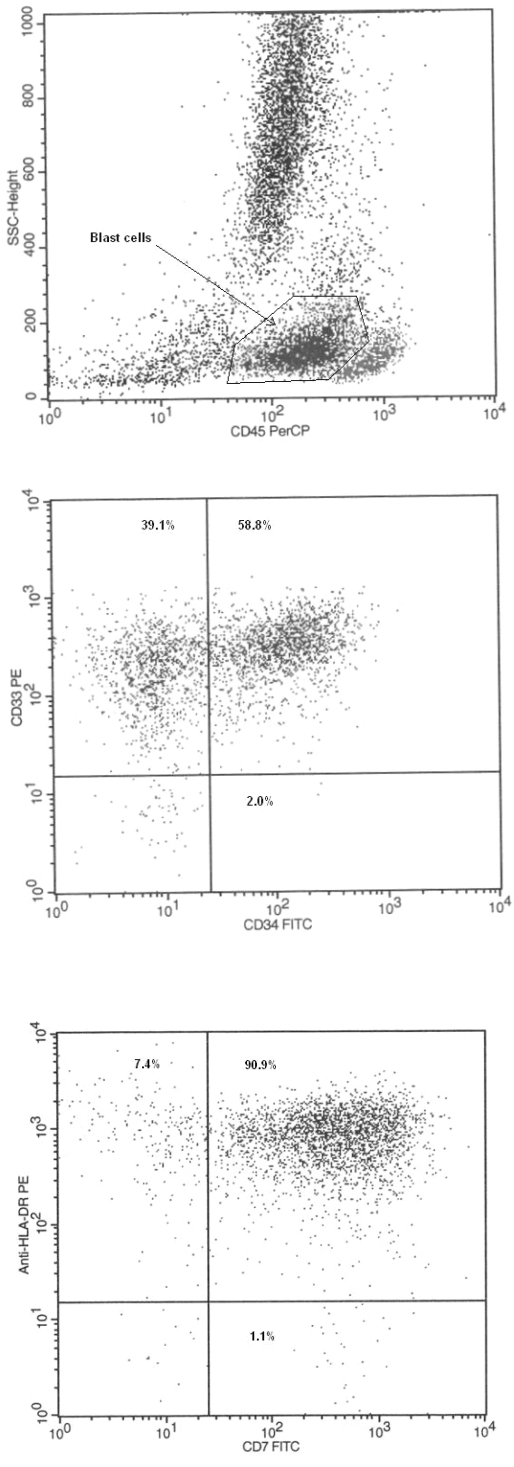

Fig. 1. Dot plots of CD7/CD34-positive AML patients

Table 2. **Antigen expression**

	HLA-DR	CD2	CD5	CD7	CD10	CD13	CD14	CD19	CD33	CD34	CD56	CD7/CD34
Patients, N	42	42	42	42	42	42	42	42	42	42	42	42
Positive patients, n, (%)	37 (89%)	8 (18%)	2 (4%)	24 (57%)	4 (10%)	40 (95%)	12 (29%)	7 (16%)	38 (91%)	29 (68%)	9 (22%)	22 (52%)

tive in 29% and CD56 in 22% of patients. The combined CD7/CD34 phenotype was present in 52% of patients. (Fig. 1).

Relationship between immunophenotype and treatment outcome

Of the 42 patients, 26 (61%) achieved complete remission. In patients with CD7/CD34-positive immunophenotype the CR rate was 41%. In contrast, in patients who did not express CD7/CD34 immunophenotype the CR rate was 81% (P = 0.01). Disease-free survival and overall survival of patients expressing CD7/CD34 immunophenotype also differed significantly from those in patients who did not express CD7/CD34 combination: for 1 year DFS 5% *vs* 44% (P = 0.01), for 1 year OS 27% *vs* 62% (P = 0.02). The results are presented in Table 3.

Table 3. **Relationship between immunological phenotype and treatment outcome**

Markers	Patients, n	CR, %	1-y DFS, %	1-y OS, %
CD7/CD34 pos.	22	41	5	27
CD7/CD34 neg.	20	81	44	62
P value		0.01	0.01	0.02

Relationship between other clinical and biological parameters and treatment outcome

The other factors influencing achievement of complete remission, 1-year disease-free survival and 1-year overall survival are summarized in Table 4.

One-year DFS and 1-year OS significantly decreased with age. one-year DFS significantly decreased also with increasing the WHO performance status >2. Complete remission rate, one-year DFS and 1-year OS were not associated with the other variables.

Prognostic value

We have included in this prognostic value the CD7/CD34 positive phenotype.

The patients were pooled depending on the presence or absence of this phenotype. The estimated 1-year DFS and 1-year OS are shown in Figs. 2 and 3, respectively.

DISCUSSION AND CONCLUSIONS

The results concerning the prognostic implication of surface antigen expression in AML have been controversial (8–12). However, the comparability of the results can be hampered by methodologic differences in the detection of antigen expression as well as by differences in the patient population studied and treatment regimens administered (8–12). Our study involved immunophenotyping examinations in addition to several clinical and biological parameters in adults with newly diagnosed AML.

Our results do not indicate that the expression of one antigen can be applied for risk stratification in adult AML at diagnosis. In fact, the majority of markers are associated with both poor and good prognostic factors. AML patients expressing one antigen do not comprise a sole biological entity of AML but present a heterogeneous group. However, the sub-

Table 4. **Factors influencing achievement of complete remission, one-year disease-free survival and one-year overall survival**

Variable	Patients, n	CR, %	1-y DFS, %	1-y OS, %
		Age		
<60	30	68	32	54
>60	12	50	0	10
P value		NS	0.01	0.04
		WHO performance status		
<2	26	64	41	55
>2	16	50	0	25
P value		NS	0.01	0.06
		P gp expression		
Positive (+)	22	55	22	33
Negative (–)	20	70	30	50
P value		NS	NS	NS
		LDH u/l		
< 1000	16	61	34	54
> 1000	26	56	32	51
P value		NS	NS	NS
		WBC count $\times 10^9$/l		
< 30	24	62	31	52
> 30	18	54	28	38
P value		NS	NS	NS

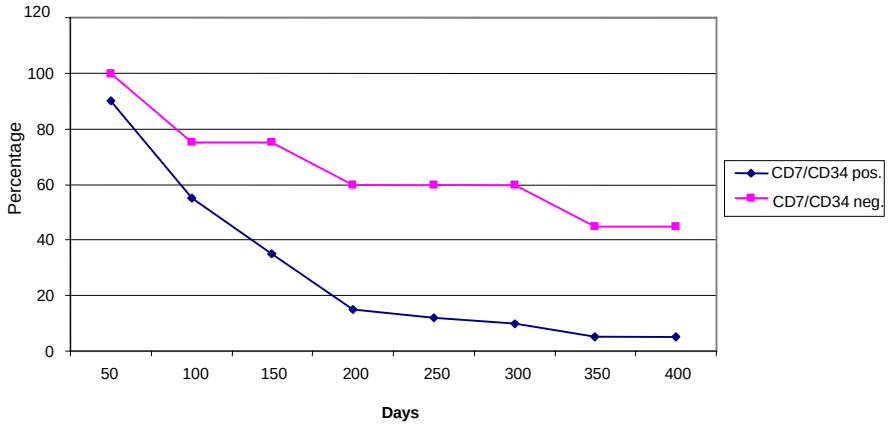

Fig. 2. Days from complete remission

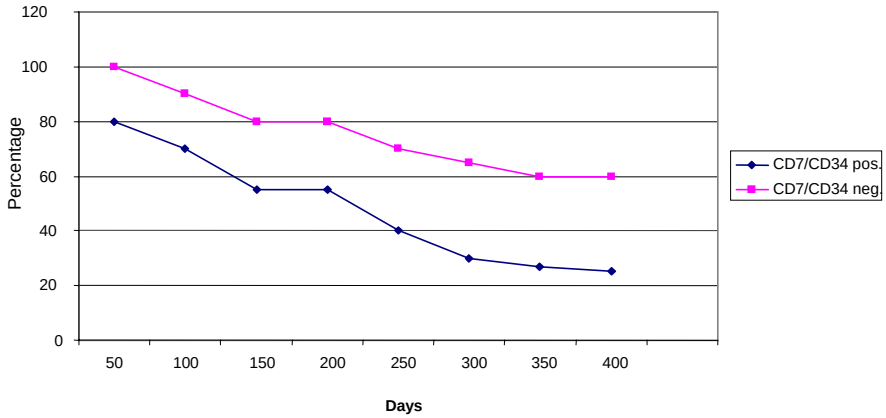

Fig. 3. Days from diagnosis

group of patients expressing a combination of several markers, such as CD7/CD34 in our study, could be recognized as a more homogeneous biological entity of AML. This combination was not associated with age, performance status, Pgp expression or any other biological and clinical variable.

Beside the CD7/CD34 phenotype, other independent prognostic factors identified in this study included older age and higher performance status, which completely correspond with well-established data from the current series of patients. The not significantly different CR rate obtained in the patients older than 60 years and patients with WHO>2 performance status could be explained by a rather similar remission induction treatment and by high standards of supportive therapy.

The Pgp expression rate was not associated with a poorer prognosis in our study. However, in accordance with other publications there was a strong relationship between Pgp and CD34 expression, which could influence the prognosis of AML patients (14). Pgp activity studies are necessary to confirm this statement.

In conclusion, the expression of CD7/CD34 phenotype is an independent prognostic factor in patients with adult AML. Patients who express this phenotype probably define a relatively homogeneous entity of AML. The results from this study will be used to help develop treatment strategies based on the risk factors of an individual patient.

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SUAUGUSIØJØ ÛMIOS MIELOGENINËS LEUKEMIJOS FENOTIPAS IR JO PROGNOZINË VERTË

Santrauka

Ávadas. Suaugusiøjø ûmios mielogeninës leukemijos gydymo efektyvumui ir iðgyvenimui prognozuoti yra siûlomi ávairûs klinikiniai ir biologiniai þymenys, tarp jø ir leukemijos imunofenotipas. Taèiau klinikiniai tyrimai atskleidë prieðtaringus rezultatus. Mes ávertinome skirtingø imunofenotipø prognozinæ reikðmæ ir jø átakà gydymo efektyvumui. Taip pat ávertinome kitø klinikiniø ir biologiniø þymenø átakà gydymo efektyvumui ir iðgyvenimui taikant chemoterapijà.

Medþiaga ir metodai. Kombinuoto CD7/CD34 imunofenotipo prognozinë reikðmë buvo iðtirta 42 suaugusiems, sergantiems ûmia mielogenine leukemija, naudojant vienodà monokloniniø antikûnø rinkiná. Platus mieloidiniø blastø imunofenotipavimas atliktas tëkmës citometrijos metodu.

Rezultatai. Ligoniai, kuriems buvo nustatytas teigiamas kombinuotas CD7/CD34 imunofenotipas, pasiþymëjo gerokai maþesniu pilnø remisijø skaièimi (P = 0,01), berecidyviniu iðgyvenimu (P = 0,01) ir bendru iðgyvenimu (P = 0,02). Kiti veiksniai, turëjæ átakos pilnø remisijø skaièiui, vieneriø metø berecidyviniam ir vieneriø metø bendram iðgyvenimui, buvo ligoniø amþius ir fizinë bûklë.

Išvados. Mes siûlome naudoti kombinuotà CD7/CD34 imunofenotipà kaip nepriklausomà prognozës rodiklá. Ðis rodiklis leidþia suskirstyti ûmia mielogenine leukemija sergantèius ligonius á rizikos grupes ir numatyti jø prognozæ, kartu ápareigoja taikyti adekvaèias gydymo schemas atsiþvelgiant á rizikos veiksnius.

Raktaþodþiai: ûmi mielogeninë leukemija, imunofenotipas, CD7/CD34 fenotipas, prognozës rodiklis