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Original research article

Enhanced pretreatment CD25 expression on peripheral blood CD4+ T cell predicts shortened survival in acute myeloid leukemia patients receiving induction chemotherapy

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ABSTRACT

Background: Recently, identification of CD25 (interleukin-2 receptor alpha) expression on leukemic blasts was correlated to early treatment failure and unfavorable outcome in acute myeloid leukemia (AML) patients. Here we wished to determine whether quantification of CD25 on peripheral blood CD4+ T cells could improve prognostication in newly diagnosed AML patients.
Methods: The mean fluorescence intensity (MFI) of CD25 expression and frequencies of peripheral blood CD4+ T cells with varying levels of CD25 and CD127 expression were assessed by flow cytometry in all studied individuals.
Results: Using univariate (unadjusted) and multivariate (adjusted) analyses we demonstrated that detection of high pretreatment CD25 expression on circulating CD4+ T cells was associated with significantly decreased survival rate of AML patients subjected to standard induction chemotherapy. These associations held true for both entire group of analyzed AML patients and different subgroups of patients identified by presence or absence of favorable and adverse molecular prognostic factors.
Conclusions: Our data indicate that quantification of CD25 expression on peripheral blood CD4+ T cells could become a novel, easily accessible method of shortened survival prognostication of AML patients subjected to standard cytotoxic therapy.

Introduction

Due to prognostic heterogeneity of currently used cytogenetic and molecular markers, the search for novel prognostic factors in acute myeloid leukemia (AML) patients is still warranted. Recently, CD25 expression on leukemic blasts was demonstrated to independently predict early treatment failure in AML patients [1]. Similarly, previous studies indicated that high expression of CD25 on leukemic blasts was a valuable predictor of outcome and residual disease in AML patients. [2,3] However, in steady-state conditions, CD25 (also referred to as interleukin-2 receptor alpha) is mainly expressed by peripheral blood T cells, predominantly CD4+ T cells [4–8]. Initially, CD25 was considered a marker of regulatory T cells that were characterized by CD4+CD25+ phenotype [9–12] Further studies demonstrated that CD25 is not exclusively expressed by Treg cells and it is substantially expressed by activated/effector/memory CD4+ T cells [4,8,13–15]. To date, significance of assessment of CD25 expression on circulating CD4+ T cells was not investigated in hematological malignancies.

Here we quantified levels of CD25 expression on peripheral blood CD4+ T cells in newly diagnosed AML patients. Furthermore,

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we tested whether measuring of CD25 expression on circulating CD4+ T cells could improve prognostication of shortened survival or treatment response.

Materials and methods

Patients

To this point, we recruited 46 patients diagnosed with acute non-promyelocytic myeloblastic leukemia (Table 1) who further became severely neutropenic following introduction of chemotherapy. Their mean age at the time of sample collection was age was 61.7 years. There were sixteen female and thirty male patients. Patients with acute promyelocytic leukemia and those AML patients who received corticosteroids at the beginning of the treatment course had been excluded from the study. Diagnoses were established in accordance with the WHO classification system [16]. Blood counts and flow cytometry were performed in order to confirm the presence of blast cells. Cytogenetic and molecular assays were used to identify several risk groups (AML1/ETO, CBFβ/MYH11, MLLT3-MLL and frequently mutated genes FLT3-ITD, NPM1, CEBPA) as recommended by WHO guidelines. Based on the above analyses, the risk pattern of AML patients was determined as following: 3 patients (6.52%) were classified as "good-risk" patients (t(8;21)), 21 subjects (45.65%) had intermediate risk (diploid karyotype features with 4 internal tandem duplication of Fms-like tyrosine kinase 3 (FLT3-ITD), 3 patients with t(9;11) and 14 with different abnormalities not assigned to either good or bad risk group) and 18 subjects (39.13%) were classified as unfavorable-risk group (with del(5q), del(7q), or complex (≥3) abnormalities).

All patients were screened for the presence of an infection site using clinical and microbiological data at the time of diagnose. Patients who received corticosteroids or antibiotics at the beginning of the treatment course were excluded. At study entry, a complete medical history was taken and vital signs were determined; blood and urine specimen were obtained for laboratory testing and a chest X-ray was performed. Cultures of blood, urine and biological material from any suspected site of infection were obtained. Maximum body temperature and absolute neutrophil counts were recorded daily during the study period. A complete evaluation of the clinical status of each patient, including an assessment of adverse events was performed. All patients had a central venous access and were during induction therapy on oral anti-bacterial (sulfametoksazol + trimetoprim) and anti-fungal (nystatin) prophylaxis.

AML patients were treated in the Department of Hematology of the Medical University of Bialystok from 2011 to 2013 with induction chemotherapy regimens corresponding to the standard therapy based on the Polish Adult Leukaemia Group. For patients under 60 years ($n = 27$) the seven days induction chemotherapy was given: cytarabine was delivered as a continuous IV infusion for seven consecutive days at a dose of 200 mg/m^2, while anthracycline for three consecutive days as an IV push at a dose of 50 mg/m^2, cladribine was administered for five days as an IV push at a dose of 5 mg/m^2 (DAC schedule) [17]. For patients above 60 years ($n = 19$) the cytarabine was reduced to 100 mg/m^2.

The first neutropenic febrile episode was treated with meropenem (3 g/1.0 g every 8 h) and amikacin (1.0 g per day). If the patient remained febrile at 48 h despite first line treatment, glycopeptides (30 mg/kg vancomycin) were administered. If patients did not respond to this therapy after next 48 h, the computer tomography of the chest was performed to evaluate the possible fungal lung infection. If diagnosed, fungal infection in all cases was treated with caspofungin (70 mg first dose followed by 50 mg *iv*).

After induction chemotherapy, the morphology response was evaluated following the recommendation by Chason et al. [18]. Fifteen patients achieved complete remission (CR) after induction and nineteen patients were classified as non-responders (NR). Twelve patients died during the applied chemotherapy and therefore they were further referred to as D.

Table 1
Clinical and molecular characteristic of studied patients.

	Clinical and molecular characteristics of patients						
	All AML patients		AML patients with low or high CD25 expression				*p* value
	n = 46		Low CD25 MFI *n* = 23		High CD25 MFI *n* = 23		
Age at diagnosis							
Mean (±SD)	61.7 ± 16.69		57.4 ± 21.2		66 ± 10.05		0.5319[a]
	Clinical and molecular characteristics of patients						
	All AML patients		AML patients with low or high CD25 expression				*p* value
	n = 46		Low CD25 MFI *n* = 23		High CD25 MFI *n* = 23		
	n	%	*n*	%	*n*	%	
Sex							
Female	16	34.78	7	30.43	9	39.13	0.7575[b]
Male	30	65.21	16	69.56	14	60.86	
Cytogenetics							
Good	3	6.52	3	13.04	0	0	0.3511[b]
Intermediate	21	45.65	10	43.47	11	47.82	
High	18	39.13	9	39.13	9	39.13	
ETO mutation	3	6.52	3	13.04	0	0	0.2334[b]
FLT3 mutation	4	8.69	1	4.34	3	13.04	0.6105[b]
Treatment response							
Complete remission (CR)	15	32.6	11	47.82	4	17.39	0.0118[b]
Non-responders (NR)	19	41.3	10	43.47	9	39.13	
Death (D)	12	26.08	2	8.69	10	43.47	

Low or high CD25 MFI refers to values either lower or higher than median CD25 expression observed in all AML patients at the time of diagnosis.
SD – standard deviation; ETO mutation – eight twenty one (t8/21); FLT3 mutation – internal tandem duplication of Fms-like tyrosine kinase 3.
[a] Mann–Whitney *U* test was used.
[b] Fisher's exact test was used.