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ORIGINAL ARTICLE

Biological implications of circulating CD34$^+$ cells in myelodysplastic syndromes

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Summary

Purpose: *To evaluate the biological and clinical significance of circulating CD34$^+$ cells in patients with myelodysplastic syndromes (MDS).*

Methods: *The relative count of CD34$^+$ cells in peripheral blood was evaluated by flow cytometry and the results were recorded on the total number of mononuclear cells (MNCs). CD34$^+$ status was correlated with the percentage of circulating and bone marrow blasts, cytogenetic studies, CFU-GM colony growth, overall survival and transformation to acute myeloid leukemia (AML).*

Results: *The number of MNC positive for anti-CD34 monoclonal antibody in the healthy control group ranged from 0.00% to 0.73%. Therefore, the cutoff value for overexpression of CD34 antigen on peripheral blood MNC of MDS patients was ≥1% (CD34$^+$ cases). The mean number of circulating CD34$^+$ MNCs in 30 MDS patients was significantly higher than in the control group (p=0.009). The proportion of circulating CD34$^+$ MNCs did not correlate with the blast count in the peripheral blood (r=0.282, p=0.131), neither with the blast count in the bone marrow. In contrast, the proportion of circulating CD34$^+$ cells in MDS patients was significantly correlated with the proportion of bone marrow CD34$^+$ cells (r=0.461, p=0.035). The proportion of circulating CD34$^+$ cells did not correspond to the percentage of blast count in the bone marrow, neither with the presence of cytogenetic abnormalities or abnormal growth of GM-progenitors. The median actuarial survival of 19 patients with elevated proportion of circulating CD34$^+$ cells was 16 months, as compared to >57 months in 11 patients with CD34$^+$ cells within normal range (p=0.16). Five patients with elevated proportion of circulating CD34$^+$ cells progressed to AML, as compared to only one of CD34-negative (CD34$^-$) cases.*

Conclusion: *The presence of circulating CD34$^+$ cells is a common finding in MDS, but no significant correlations with clinical and/or biological features of the disease have been found.*

Key words: biology, CD34$^+$ cells, myelodysplastic syndromes, peripheral blood, prognosis

Introduction

MDS are malignant disorders of hematopoietic cells. For many neoplasms, immunophenotypic data of the neoplastic cells provide valuable information in clinical practice. However, the clinical relevance of the immunophenotypic data has not yet been firmly established for MDS [1]. As CD34 expression is a marker of hematopoietic stem cell (HSC), experiments have focused on the CD34$^+$ cell abnormalities in patients with MDS [2]. Myelodysplastic and normal CD34$^+$ progenitor cells show a number of differences, including increased coexpression rate of CD13 antigen and lineage commitment to non erythroid growth with impaired differentiation [3], aberrant response to stimulation with growth factors [4,5] and growth advantage of myelodysplastic CD34$^+$ cells over normal progenitor cells [6]. A number of genes expressed in myelodysplastic CD34$^+$ marrow cells has shown more than 5-fold downregulation or upregulation compared to healthy subjects [2], the ratio of proapoptotic Bax/Bad to antiapoptotic Bcl-2/Bcl-X_L is higher in MDS CD34$^+$ cells than in normal cells [7], all suggesting important biological differences between the clonal and normal progenitor pool.

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The prognostic value of CD34 expression on bone marrow cells in MDS patients has been shown in a number of studies [8-10]. In contrast, there are only few reports on the association of circulating $CD34^+$ cells and prognosis in patients with MDS [11]. Therefore, the aim of this study was to evaluate the biological and clinical significance of circulating $CD34^+$ cells in patients with MDS.

Methods

Patients

Our study included 30 adult patients with primary MDS, diagnosed according to the FAB Cooperative Group criteria [12] in the Clinical Center of Serbia, during 1990-1999. All patients met the minimal diagnostic criteria for MDS, recommended by Reizenstein and Ost [13]. Patients with secondary and therapy-related MDS were excluded. The patients were evaluated for AML transformation and overall survival. Overall survival was defined as the time from the date of the MDS diagnosis until death from any cause, and observation ended at the time of the last contact with the patients last known to be alive.

Immunophenotyping

Cell surface membrane antigen analysis was performed on heparinized peripheral blood specimens by using indirect immunofluorescence and flow cytometry methods (EPICS-C, Coulter® or FACScalibur, Becton Dickinson®), applied on MNCs, as previously described [14]. A commercial mouse anti-human monoclonal antibodies against CD34 (HPCA-2; Becton Dickinson) was used as primary antibody. GAM-FITC (Bekman Coulter or Becton Dickinson) was used as a second step reagent. Results were recorded on MNCs population, gated according to intermediate forward-light/orthogonal light scatter properties [15]. Antigen expression was given as a proportion of labeled cells in a studied population and compared to antigen expression of the same population in the peripheral blood of the control group (10 healthy individuals).

Cytogenetic studies

Cytogenetic analysis was performed at diagnosis on unstimulated bone marrow cells by direct preparation and following 24h in culture in RPMI 1640 medium with 25% fetal calf serum at 37°C. The HG-banding method [16] was used for harvesting and preparation of the metaphase and interphase slides. The karyotype was interpreted according to the International System for Human Cytogenetic Nomenclature [17].

Colony assays

In vitro cell culture studies were done at referral, according to the method described by Iscove et al. [18] and Messner et al. [19]. A culture containing less than 2 colonies (CFU-GM) and/or less than 20 GM-clusters was considered a "no growth". A culture containing low colony and high cluster number or normal/high colony and high cluster number was judged as a "leukemic" growth pattern. All other cultures were considered "normal" growth of GM-progenitors [20].

Statistical analysis

Computer-aided analysis was done with methods of descriptive statistics. The correlations between different parameters were assessed by linear regression test. Survival and AML development were plotted according to the Kaplan-Meier method and curves were compared by using log-rank test. All statistical tests were two-sided and a p-value <0.05 was considered as statistically significant.

Results

Among 30 patients enrolled in this study, there were 18 males and 12 females (median age 63 years, range 32-84). According to FAB classification, there were 8 patients with RA, 3 with RARS, 8 with RAEB, 6 with RAEBT and 3 patients with CMML.

The number of MNCs positive for anti-CD34 monoclonal antibody in the control group ranged from 0.00 to 0.73%. Therefore, the cutoff value for overexpression of CD34 antigen on peripheral blood MNCs of MDS patients was ≥1% ($CD34^+$ cases). The mean number of circulating $CD34^+$ mononuclear cells in 30 MDS patients was significantly higher than in healthy persons (Table 1). Eleven of 30 tested patients have had <1% MNCs in the peripheral blood reactive with the monoclonal antibody HPCA-2, which corresponded to the values found in the control group.

The proportion of $CD34^+$ cells in the peripheral blood was partly correlated with FAB classification, since the median expression was highest in RAEBT (8%), as opposed to the expression in other FAB subtypes: RA (1.5%), RARS (1.0%), RAEB (2.5%) and CMML (3%). The proportion of circulating $CD34^+$ MNCs cells did not correlate with the blast count in pe-

Table 1. Expression of CD34 antigen on bone marrow and peripheral blood mononuclear cells in patients with MDS and control group (results are given as a percent of mononuclear cells reactive with HPCA-2)

	MDS	*Control group*	*p-value*
Peripheral blood			
x ± SEM	4.73±1.0	0.22±0.11	0.009
Bone marrow			
x ± SEM	6.80±1.35	4.25±0.75	NS

NS: not significant, SEM: standard error of the mean, x: mean value

ripheral blood (r=0.282, p=0.131), neither with the blast count in the bone marrow. In contrast, the proportion of circulating $CD34^+$ cells in MDS patients was significantly correlated with the proportion of bone marrow $CD34^+$ cells (r=0.461, p=0.035). The proportion of circulating $CD34^+$ cells did not correspond to the percentage of the blast count in bone marrow, neither with the presence of cytogenetic abnormalities or abnormal growth of GM-progenitors (Table 2).

Patients with higher proportion of circulating $CD34^+$ cells (CD34 positive cases) and patients with circulating $CD34^+$ cells within normal range (CD34 negative cases) were compared regarding overall survival and risk for leukemic transformation. Eleven of 19 $CD34^+$ cases died during the follow-up period, in contrast to 4 of 11 $CD34^-$ cases (p>0.05). The median actuarial survival of $CD34^+$ cases was 16 months, as compared to >57 months in $CD34^-$ cases (p=0.16; Figure 1). Five patients with circulating $CD34^+$ cells developed AML, as compared to only one $CD34^-$ cases, but the small number of cases in both groups ($CD34^-$ vs. $CD34^+$ cases) made the statistical analysis impossible.

Table 2. Proportion of $CD34^+$ cells in the peripheral blood related to the percentage of bone marrow blasts, karyotype and the type of CFU-GM colony growth (results are given as a percent of mononuclear cells reactive with HPCA-2)

	x ± SD	*Median*	*Range*	*p-value*
Bone marrow blasts (%)				
<5	4.93±7.11	1.5	0-11	0.78
≥5	4.27±3.7	3	0-27	
Karyotype				
Normal	3.06±3.28	2.0	0-13	0.16
Abnormal	6.3±8.4	7.0	0-27	
GM-progenitor growth				
Normal	6.07±7.6	3.5	0-27	0.41
Abnormal	3.33±3.1	1.0	0-11	

SD: standard deviation, x: mean value

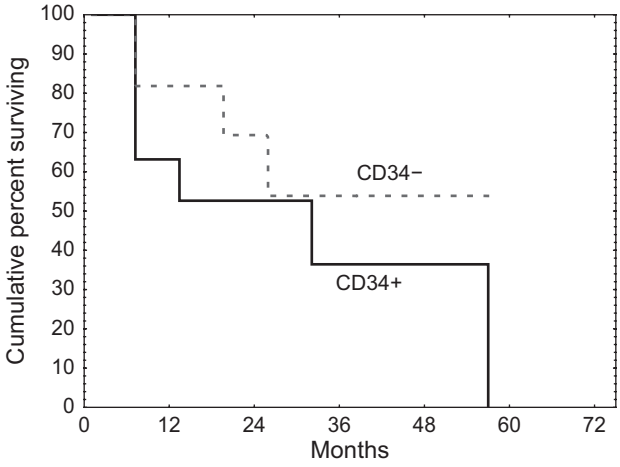

Figure 1. Kaplan-Meier plots predicting overall survival: CD34 positive cases compared to CD34 negative cases. The difference was not statistically significant (p=0.16).

Discussion

We found significantly higher number of circulating $CD34^+$ cells in the MDS group in comparison with the control group. Sixty-three percent, or 19 out of 30 patients tested, had ≥1% $CD34^+$ cells as a percentage of their peripheral blood MNCs, as compared with 37% cases in the study by Sullivan and colleagues [11]. This difference could be attributed to the larger proportion of low risk (RA, RARS) patients in the aforementioned study as compared with our group of MDS patients (63% and 43% of the cases, respectively).

The proportion of circulating $CD34^+$ cells was higher in advanced disease (RAEBT, RAEB, CMML) compared with our low risk MDS patients. This finding was in accordance with previous reports [11,21]. Moreover, highly sensitive flow cytometry disclosed a distinct difference in the size of circulating $CD34^+$ cells between RA and RAEB (T) patients. Namely, the $CD34^+$ cells of RA patients are distributed mainly in the low forward scatter (FSC) (lymphocyte region) in contrast to $CD34^+$ cells of RAEB (T) patients which are observed in high FSC (blast region) [21]. Such differences imply a diverse biological origin of circulating $CD34^+$ cells in low risk and high risk patients.

We did not establish any correlation between the proportion of circulating $CD34^+$ cells and blast count in the peripheral blood. However, processing of the sample for flow cytometric analysis complicates the comparison between the visual blast count and the CD34 value [22]. Moreover, errors in the visual estimation of blast cells in the peripheral blood are expected in the case of presence of microblasts or hematogones, due to the heterogeneity of blast cells in MDS. In contrast, the proportion of circulating $CD34^+$ cells in our MDS patients

was significantly correlated with the proportion of bone marrow $CD34^+$ cells, suggesting altered adhesive interactions between clonogenic hematopoietic stem cells and the underlying marrow stroma (or endothelium) and increased angiogenesis in advanced disease.

Fuchigami and colleagues [21] showed that high absolute number of circulating $CD34^+$ cells ($\geq 1.0 \times 10^9$/L) is a poor prognostic factor in MDS. Similarly, Sullivan and coworkers [11] showed significant association between the presence of $CD34^+$ cells in the peripheral blood and short survival and transformation to leukemia, and concluded that this finding is better prognostic indicator than cytogenetic studies or CFU-GM colony growth. On the contrary, the proportion of circulating $CD34^+$ cells did not correspond with either the presence of cytogenetic abnormalities or abnormal growth of GM-progenitors, or with the outcome of our patients.

The present study showed that the presence of circulating $CD34^+$ cells is a common finding in MDS. This observation should be kept in mind when autologous peripheral blood stem cell transplantation (SCT) is considered as a therapeutic approach for patients with MDS, regardless of efficient *in vivo* purging and harvest of sufficient number of autologous stem cells. Moreover, it was recently shown that the most immature hemopoietic cells in MDS (CD45-, CD34-, CD38-, Lin-) had the same cytogenetic aberration like the myeloblasts [23]. Autologous SCT after successful induction chemotherapy may increase the proportion of long-term survivors, but the results for older or the majority of patients are unsatisfactory: the relapse rate is up to 75%, and the 2-year probability of disease free survival is only 25% for patients aged 40-60 years [24]. Therefore, there is very limited enthusiasm for the future of autologous SCT in the management of MDS patients [24].

We conclude that there is no significant correlation between the presence of circulating $CD34^+$ cells and the clinical and/or biological features of the disease. Therefore, further immunophenotypic studies are needed to determine the precise implications of the circulating $CD34^+$ cells in MDS which will hopefully improve the clinical approach to these intractable disorders.

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