Brief report

Hematogones: a new prognostic factor for acute myeloblastic leukemia

Sylvain P. Chantepie,[1,2] Véronique Salaün,[3] Jean-Jacques Parienti,[2,4-6] Florence Truquet,[3] Margaret Macro,[1] Stephane Cheze,[1] Jean Pierre Vilque,[7] and Oumedaly Reman[1]

[1]Service d'Hématologie Clinique, Centre Hospitalier Universitaire, Caen, France; [2]Université de Caen, Basse-Normandie, France; [3]Laboratoire d'Hématologie, Centre Hospitalier Universitaire, Caen, France; [4]Département de Biostatistiques et de Recherche Clinique, Centre Hospitalier Universitaire, Caen, France; [5]Inserm, U 707, Paris, France; [6]Université Pierre et Marie Curie, Paris 6, UMR-5 707, Paris, France; and [7]Service d'Hématologie, Centre François Baclesse, Caen, France

Acute myeloid leukemia (AML) patient outcomes remain heterogeneous, and new prognostic tools are needed to assess the risk of relapse. Hematogones (HGs) are normal B-lymphocyte precursors, which increase in number in hematologic diseases. The prognostic impact of the presence of detectable HGs on the leukemia-free survival (LFS) and overall survival of 120 consecutive patients with AML in first complete remission was investigated by flow cytometry. Patients who had HG levels more than 0.01% had a significantly better median LFS (29.2 vs 11.7 months; $P = .001$) and overall survival (not reached vs 23.5 months; $P = .011$). According to Cox analysis, an HG level more than 0.01% was an independent predictor of LFS (hazard ratio = 0.5; 95% confidence interval, 0.28-0.90, $P < .03$) when age, leukocytosis, the number of chemotherapy cycles, and the standardized cytogenetic and molecular risk subgroups were controlled for. These results indicate that HG analysis may help to define the risk of relapse in AML patients. (*Blood*. 2011;117(4):1315-1318)

Introduction

The prognosis of acute myeloid leukemia (AML) patients can be estimated based on disease-related and patient-specific factors, such as karyotype abnormalities.[1] Despite these risk factors, the prognosis of patients within each AML subgroup remains heterogeneous. Several studies have identified biologic factors that are associated with AML outcome, although the relevance of these factors has not been tested in prospective studies.[2-5]

Hematogones (HGs) are normal B-lymphocyte precursors that are present in the bone marrow of healthy persons.[6] HGs are typically identified by their morphologic features and a distinct flow cytometric immunophenotype.[7,8] Although high HG levels are common in healthy infants,[9] in patients with lymphoma or myelodysplastic syndromes, and patients undergoing bone marrow regeneration subsequent to chemotherapy or bone marrow transplantation,[8,10] the number of HGs declines with age or bone marrow involvement with neoplastic cells. HGs are often identified by 4-color flow cytometry with coexpression of CD10 and CD19 and low-intensity CD45 expression. However, the prognostic significance of the number of HGs in acute myeloblastic leukemia has not been explored. The goal of this study was to use flow cytometry to evaluate the number of HGs in AML patients in complete remission and to assess the prognostic impact of HG levels on leukemia-free survival (LFS) and overall survival (OS).

Methods

Study design

From January 1999 to December 2009, a total of 120 consecutive patients with AML in first complete remission (according to the International Working Group criteria[11]) were enrolled in this study. The study was granted Institutional Review Board approval from the Centre Hospitalier Universitaire (Caen, France), and the committee waived the informed consent requirement because of the observational nature of the protocol. The patients were classified according to their standardized cytogenetic and molecular risk subgroups.[12] Samples were obtained by bone marrow aspiration after hematologic recovery after chemotherapy. All of the patients were treated with a 3 + 7 chemotherapy schedule, and most of theses schedules adhered to Acute Leukemia French Association protocols.[13,14]

Flow cytometric methods

Cells were derived from fresh bone marrow and were prepared for flow cytometry as previously described.[8] Two 4-color combinations were used for each assay: CD10/CD19/CD20/CD45 and CD10/CD22/CD38/CD45. Up to 30 000 cells were acquired per sample, and the data were visualized using a plot of side scatter scale versus CD45 staining. The data were collected using a FACSCanto II flow cytometer (BD Biosciences). A multiparametric analysis of antigen expression was performed with FACSDiva Software (BD Biosciences). The combination of CD10/CD19 coexpression and low-intensity CD45 expression was considered to be indicative of HGs; the sensibility cut-off was 0.01% of the total number of nucleated cells.[8] The HG-positive group was defined as patients who had more than or equal to 0.01% HGs in the bone marrow aspiration sample.

Statistical analysis

The patients' characteristics were compared with a χ^2 test for binary variables and a Mann-Whitney test for continuous variables. Survival was plotted with Kaplan-Meier curves, and the data for the various groups were compared with a log-rank test. Multivariate analysis was performed with a

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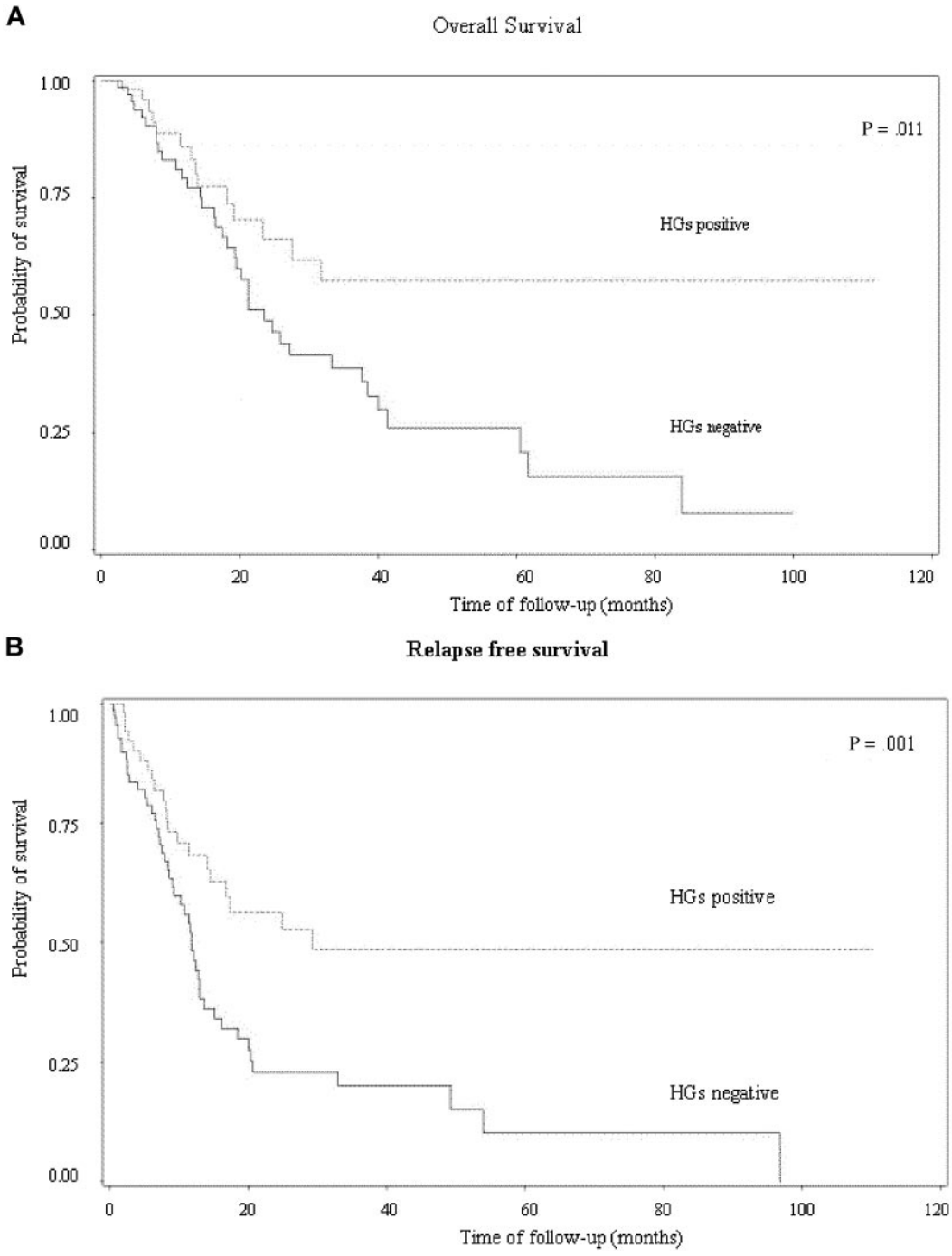

Figure 1. Kaplan-Meier survival curves. OS (A) and relapse-free survival (B) according to HG status.

Cox model after the proportional hazard assumption was checked. A *P* value less than .05 was considered to be statistically significant.

Results and discussion

The baseline characteristics of the 120 patients who where included in the study are summarized in supplemental data (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

The median follow-up time was 18 months, and 68 patients (57%) relapsed after a median of 8.4 months. Twenty-five patients (21%) underwent allogeneic hematopoietic stem cell transplantation.

The median and mean percentages of total HGs were 0% (interquartile range, 0.00-0.55) and 0.77% (SD = 2.18), respectively. Fifty-six patients (46%) had detectable HGs in the bone marrow after induction therapy. Twenty-six patients were younger than 50 years, 12 patients were 50 to 60 years old, and 18 patients were more than 60 years old. No detectable HGs were found in 64 of the bone marrow samples: 37 (49.3%) in the group of patient younger than 60 years and 27 (60%) in the older group (*P* = not significant).

As shown in Figure 1, AML patients in first complete remission who were positive for HGs in the bone marrow had a significantly

Table 1. Impact of prognostic factors on relapse-free survival and OS

Prognostic factor	Univariate analysis: *P*	Multivariate analysis: HR (95% CI), *P*
Relapse-free survival		
HGs	.001	0.50 (0.28-0.90), < .03
Age	.18	0.99 (0.98-1.01), .72
Chemotherapy cycle*	< .0001	2.69 (1.19-6.06), < .02
Leukocytosis	< .0001	1.08 (1.02-1.15), < .007
Risk subgroup†	< .0001‡	
Favorable		1 (referent)
Intermediate-I		3.55 (1.63-7.75), < .002
Intermediate-II		1.65 (0.73-3.72), .2
Adverse		5.28 (2.35-11.85), < .0001
OS		
HGs	.011	0.62 (0.30-1.29), .2
Age	.22	1.00 (0.98-1.02), .9
Chemotherapy cycle*	.0072	2.65 (1.06-6.60), < .04
Leukocytosis	< .0001	1.13 (1.05-1.22), .0007
Risk subgroup†	< .02‡	
Favorable		1 (referent)
Intermediate-I		2.46 (1.03-5.89), < .05
Intermediate-II		1.55 (0.62-3.86), .3
Adverse		3.51 (1.39-8.85), < .008

*Number of chemotherapy cycles required to achieve complete remission.
†Dohner et al.[12]
‡According to overall log-rank test.

higher median LFS (29.2 vs 11.7 months; $P = .001$) and OS (not reached vs 23.5 months; $P = .011$) compared with patients who were negative for HGs. Univariate analysis indicated that the leukocytosis at the time of diagnosis, the number of chemotherapy cycles required to achieve complete remission, and the standardized cytogenetic and molecular risk subgroups were prognostic factors for LFS and OS. Cox analysis indicated that the presence of HGs was independently associated with a longer LFS but was not associated with OS (Table 1).

In the present study, we assessed the significance of the presence of HGs in the bone marrow during remission after chemotherapy, and we demonstrated the prognostic impact of a detectable number of HGs on LFS. To our knowledge, this is the first report describing the incidence and prognostic value of B-cell precursors in AML patients in first complete remission. However, because of our small sample size in this study, these data will have to be confirmed in future studies with larger numbers of patients.

There are several hypotheses that can explain the presence of HGs and their prognostic impact in AML. Because HGs are normal bone marrow cells, they may reflect the quality of the bone marrow response to chemotherapy. Moreover, some studies have described a decrease in bone marrow B-cell precursors in myelodysplastic syndrome with excess blasts,[15] with bone marrow infiltration by leukemic or neoplastic cells,[8] or with the mutation of specific genes, such as NRAS.[16] The chemotherapy-mediated decrease in leukemic cells may promote HG growth and development in the bone marrow.

A previous study has shown that HGs can be detected with high incidence (> 40%) in bone marrow after chemotherapy treatment of AML.[17] Although the quantity of B-cell precursors decreases with age, HGs were identified in patients more than 50, 60, and 70 years old in the present study, and there was no statistically significant difference between these age groups. We found that HG levels equivalent to more than 0.01% of the total nucleated cells in the bone marrow after induction therapy were associated with a significantly decreased risk of relapse.

HG detection using flow cytometry as part of AML patient follow-up has many advantages. First, HG identification by cytology alone may be difficult in bone marrow aspirates after chemotherapy. Second, the HG flow cytometric phenotype is highly stable, unlike that of leukemic cells. Third, the number of cells per tube that is required for a positive HG signal is relatively low compared with the amount of bone marrow nucleated cells required (2×10^5 to 1×10^6) for minimal residual disease evaluation by flow cytometry.[3,18] Finally, flow cytometric analysis of HGs is a simple, rapid method and is suitable for clinical screening procedures. The prognostic value of the presence of detectable HGs should be further studied in larger groups of patients in future studies to verify these results.

Authorship

Contribution: S.P.C., O.R., and J.-J.P. designed the project and directly participated in drafting, writing, and editing of the manuscript; V.S. and F.T. directed the flow cytometric and data analysis; M.M., S.C., and J.-P.V. provided patient samples; J.-J.P. carried out the statistical analysis; and O.R. supervised the study.

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Correspondence: Sylvain P. Chantepie, Service d'Hématologie, Centre Hospitalier Universitaire, Côte de Nacre, 14033 Caen Cedex, France; e-mail: chantepie-s@chu-caen.fr.

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