CURRENT ONCOLOGY
A Canadian Cancer Research Journal

ORIGINAL ARTICLE

Hematogones: a sensitive prognostic factor for Chinese adult patients with acute myeloid leukemia

L. Li MD,*[a] R. Fu MD,*[a] T. Zhang MD,* X. Xie MD,* J. Liu,* J. Tao PhD,* J. Song MD,* H. Liu MD,* W. Zhang MD,* W. Lu PhD,† and Z. Shao MD*

ABSTRACT

Background Hematogones (HGs) are normal B-lymphocyte precursors that increase in some hematologic diseases. Many studies indicate that HGs might be a favourable prognostic factor. We thus considered it important to determine whether HGs are also a prognostic factor for Chinese adult patients with acute myeloid leukemia (AML) and whether the HG-positive and HG-negative groups show any serologic or phenotypic differences.

Methods Chinese adult AML patients ($n = 177$) who were all initially HG-negative underwent standard chemotherapy and were thereafter divided into HG-positive and HG-negative groups according to HG levels in bone marrow during their first remission.

Results The follow-up study confirmed that survival duration (both leukemia-free and overall) was significantly greater in the HG-positive group than in the HG-negative group and was accompanied by a lower relapse rate. A retrospective study of patient characteristics at the time of first diagnosis revealed some differences between the HG-positive and the HG-negative groups, including elevations in white blood cells, lactate dehydrogenase, and β_2-microglobulin in the HG-negative group. Retrospective phenotypic analysis revealed a significantly lower proportion of abnormal chromosome karyotype and CD34 expression in HG-positive patients. Finally, we evaluated whether additional intensive chemotherapy after standard chemotherapy could further increase HGs.

Conclusions The present work verified the validity of HGs as a prognostic factor for Chinese adult patients with AML. Compared with HG-negative patients, HG-positive patients not only experienced longer survival and a lower relapse rate, but they also had some serologic and phenotypic characteristics that are all considered indicators of better outcome. Additional intensive chemotherapy could further increase the level of HGs, which might imply better clinical results.

Key Words Acute myeloid leukemia, hematogones, prognosis, standards, intensive chemotherapy

Curr Oncol. 2016 Apr;23(2):e123-e130 www.current-oncology.com

INTRODUCTION

Hematogones (HGs) in bone marrow were initially described as cells of uncertain origin and function[1]. With the arrival of flow cytometry and immunohistochemical analysis, the use of optimal antibody combinations in four-color flow cytometry was able to identify HGs in most bone marrow specimens[2]. Although HGs are generally regarded as normal B-lymphocyte precursors in bone marrow, their properties overlap those of lymphoblasts[3]. High HG levels have been observed in non-neoplastic disease such as autoimmune disorders[4] and in neoplastic diseases such as lymphomas[5].

Knowledge about the properties and biologic functions of HGs is still limited; however, accumulating data suggest that bone marrow HGs could be a good prognostic

[a] These authors contributed equally to the present work.

Correspondence to: Zonghong Shao or Lijuan Li, Department of Hematology, General Hospital of Tianjin Medical University, No. 154 Anshan Road, Heping District, Tianjin 300052 P.R.C.
E-mail: shaozonghong@sina.com or lilijuan18@arliyun.com ■ **DOI:** http://dx.doi.org/10.3747/co.23.2877

factor in acute myeloid leukemia (AML)[6–8]. Recent flow cytometry studies to quantify HGs at various time points revealed that HGs reflect prognosis not only for regeneration of post-therapy bone marrow but also for future outcome[7,8]. Although those pilot studies demonstrated that HG frequency is a reliable biomarker to aid in the decision-making process for AML treatment, some issues remain to be clarified:

- Genetic background obviously has strong effect on both the pathogenesis and outcome of AML and whether HGs are reliable prognostic factors in all races.
- A determination of any serologic or bone marrow phenotypic differences between HG-positive and HG-negative patients is needed.
- Response to chemotherapy varies significantly for the various types of AML and has been observed to be accompanied by HG variations.
- Whether intensive chemotherapy after standard chemotherapy can further increase HGs must be determined.

Understanding the answers to those questions will undoubtedly benefit clinicians.

METHODS

Patients

From March 2006 to November 2014, bone marrow samples were collected from 177 patients newly diagnosed with AML at the General Hospital of Tianjin Medical University (Table I) before any chemotherapy or immunotherapy treatment was given. The marrow samples were tested in advance of the patients being included in the study. Only patients confirmed to be absolutely HG-negative (<0.1%) were included. The research program was approved by institutional ethics committee of Tianjin Medical University, and all participants provided written consent to participate in this study.

Reagents

Antibodies used for flow cytometry analysis were purchased from BD Biosciences (San Jose, CA, U.S.A.).

Flow Cytometry Analysis of HGs

Bone-marrow HGs were analyzed by four-color flow cytometry according to previously published methods, with slight modifications[7]. Briefly, after erythrocyte lysis, a four-color combination panel was used to identify HGs. The combination consisted of CD10, HLA-DR, CD19, CD45. Other combinations of monoclonal antibodies to detect AML and aberrant leukemia blast phenotypes were used as earlier described[9]. All the monoclonal antibodies were directly conjugated to one of these fluorochromes: fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, or allophycocyanin. A minimum of 10,000 cells per sample were analyzed by fluorescence-activated cell sorting (FACSAria cell sorter and the Diva software application: BD Biosciences), and cell-type percentages were determined.

Cells that co-expressed CD10 and CD19, with low-intensity CD45 expression, were considered to be indicative of HGs. After 100,000 events were acquired by flow cytometry, the median percentage of HGs in the bone marrow of AML patients was 1% (interquartile range: 0.25%, 3.10%). Based on that median value, we divided the participants into HG-positive (≥1%) and HG-negative (<1%) groups.

TABLE I Characteristics of patients after chemotherapy for adult acute myeloid leukemia

Characteristic	Value
Patients (*n*)	177
Sex [*n* (%) men]	82 (46.3)
Age at diagnosis (years)	
Median	46
Range	20–83
FAB classification [*n* (%)]	
M0 or M1	2 (1.1)
M2	27 (15.3)
M3	35 (19.8)
M4	45 (25.4)
M5	62 (35.0)
M6	6 (3.4)
Relapsed disease [*n* (%)]	122 (68.9)
Mean follow-up (months)	22.91±21.34
Mean survival (months)	
Overall	25.58±19.86
Leukemia-free	20.53±19.37
Mean WBCs at diagnosis ($\times 10^9$/L)	28.2±47.9
Risk stratification [*n* (%)]	
Better	59 (33.3)
Intermediate	70 (39.5)
Poor	48 (27.1)
Mean bone marrow blasts at diagnosis (%)	48.3±28.8
Hematogones	
Mean result, all patients (%)	2.07±2.12
Negative group (<1.0%)	
Patients (*n*)	76
Mean result (%)	0.28±0.31
Positive group (≥1.0%)	
Patients (*n*)	101
Mean result (%)	3.42±1.89

FAB = French–American–British; WBCs = white blood cells.

Chemotherapy Protocol

All 177 adult AML patients initially underwent standard chemotherapy using the DA–IA protocol (DA: daunorubicin 45 mg/m^2 daily on days 1–3 and cytarabine 100–200 mg/m^2 daily every 12 hours on days 1–7; IA: idarubicin 9–13 mg/m^2 daily on days 1–3 and cytarabine 100–200 mg/m^2 daily every 12 hours on days 1–7). Outcome was evaluated at 3 months after chemotherapy completion. Complete remission was defined as no detectable blasts in peripheral blood, hemoglobin 100 g/L or greater (men) or 90 g/L or greater

(women), absolute neutrophil count 1.5×10^9/L, platelets 100×10^9/L or greater, less than 5% blasts in bone marrow [French–American–British (FAB) classification], and no immunophenotypic evidence of residual leukemia by flow cytometry. Partial remission was accepted when the sum of type I and type II myeloblasts in bone marrow fell between 5% and 20%, or when the peripheral blood result did not fulfil the standard for complete remission. "No remission" was defined as a negative result for all bone marrow and peripheral blood evaluations and a clinical appearance that failed to satisfy the standard of complete remission. Relapse was defined as the appearance of leukemia cells the peripheral circulation or the presence of more than 5% blasts in bone marrow.

Subsequently, 68 patients achieving remission in generally good physical condition underwent another round of intensified chemotherapy. The protocol consisted of fludarabine 30 mg/m^2 daily on days 1–5, cytarabine 2 g/m^2 daily on days 1–5, and granulocyte colony–stimulating factor 300 μg daily from day 0 until neutrophil recovery. The selection of the 68 patients for intensive chemotherapy was based entirely on their clinical presentation, and the therapies delivered conformed to the standard of care.

Statistical Analysis

Baseline clinical and biochemical features of the patients are presented as means ± standard deviation or percentages. A mean value for each variable was calculated based on all follow-up data for each patient. Those values (follow-up laboratory values) were then grouped by the HG status of the patients. A t-test was used to compare continuous variables between groups, and chi-square tests were used to compare categorical variables. The paired t-test was used to compare mean differences between baseline and end-of-follow-up data. Logistic regression analysis was used to examine the variables that might predict overall (OS) and leukemia-free (LFS) survival. Results are described as relative risks (RRS) and their 95% confidence intervals (CIS). Cox regression analysis was used to examine whether HG status might predict OS and LFS. All statistical operations were performed using the SPSS software application (version 13.0: SPSS, Chicago, IL, U.S.A.).

RESULTS

Better Outcome in HG-Positive Adult AML Patients

As Table I and Figure 1(A,B) show, LFS and OS were both significantly longer for adult AML patients who were HG-positive than for patients who were HG-negative. With respect to 1-year and 5-year relapse rates [Figure 1(C,D)], HG-positive patients experienced significantly lower relapse rates. Figure 2 demonstrates the significantly increased remission rate in HG-positive patients (93.1% vs. 65.7% in HG-negative patients) after standard chemotherapy.

Serologic and Bone Marrow Phenotypic Parameters

Some significant differences were observed between the HG-positive and HG-negative patients, including fewer white blood cells (WBCS), lower lactate dehydrogenase (LDH), and lower β^2-microglobulin (β2MG) in the HG-positive group (Figure 3). As Table II shows, the correlation of several factors with AML patient survival was evaluated. The HG level in AML patients was positively correlated with both OS and LFS in the univariate and multivariate analyses. The univariate analysis indicated that WBCS, age, β2MG, LDH, risk stratification, bone marrow leukocytosis at the time of diagnosis, and leukemia stem cell (LSC) positivity after chemotherapy all contributed to OS and LFS. However, Cox regression analysis indicated that the presence of HGS was independently associated with longer LFS and OS. Lymphatic antigen co-expression and abnormal chromosome karyotype in bone marrow samples were also compared for the HG-positive and HG-negative patients, with the result that HG-positive patients were observed to have significantly less lymphatic antigen co-expression (57.9% vs. 32.7%) and fewer abnormal chromosome karyotypes (35.6% vs. 63.1%) in bone marrow.

HG Level by FAB Classification

Because the type of AML greatly affects outcome, it is necessary to know whether patients with different types of AML have different HG levels after chemotherapy. The FAB classification was applied to all 177 patients in the cohort, and HG levels were compared by FAB classification. As demonstrated in Figure 4, HG levels varied widely in the various types of AML, with the highest HG levels being detected in patients with the M3 type of AML. Subsequently, we compared the percentages of CD34-positive cells and LSCS after standard chemotherapy in the HG-positive and HG-negative patients (remission). Because the myeloid cells of patients with the M3 type of AML always lack CD34 expression, they were excluded from this analysis. In the remaining patients, expression of LSCS and CD34 was significantly less in bone marrow from HG-positive patients (28.44% ± 20.61% vs. 45.72% ± 24.39% in HG-negative patients, and 1.12% ± 0.78% vs. 1.73% ± 1.03% in HG-negative patients respectively; Figure 5).

Further Increase in HGs with Intensive Chemotherapy

To determine whether the greatest remission was achieved after initial standard chemotherapy or whether additional intensive chemotherapy could further increase HGS, 68 patients were given intensive chemotherapy, and their bone-marrow HG levels were compared before (2.54% ± 2.30%) and after (3.52% ± 2.54%) the intensive chemotherapy. Figure 6 indicates that the additional intensive chemotherapy significantly increased HGS.

DISCUSSION AND CONCLUSIONS

Several hypotheses have already been put forward to explain the presence of HGS and their prognostic impact in AML. Because HGS are normal bone marrow cells, they might 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[10], with bone marrow infiltration by leukemic or neoplastic cells or with the mutation of specific genes such as *NRAS*[16]. That

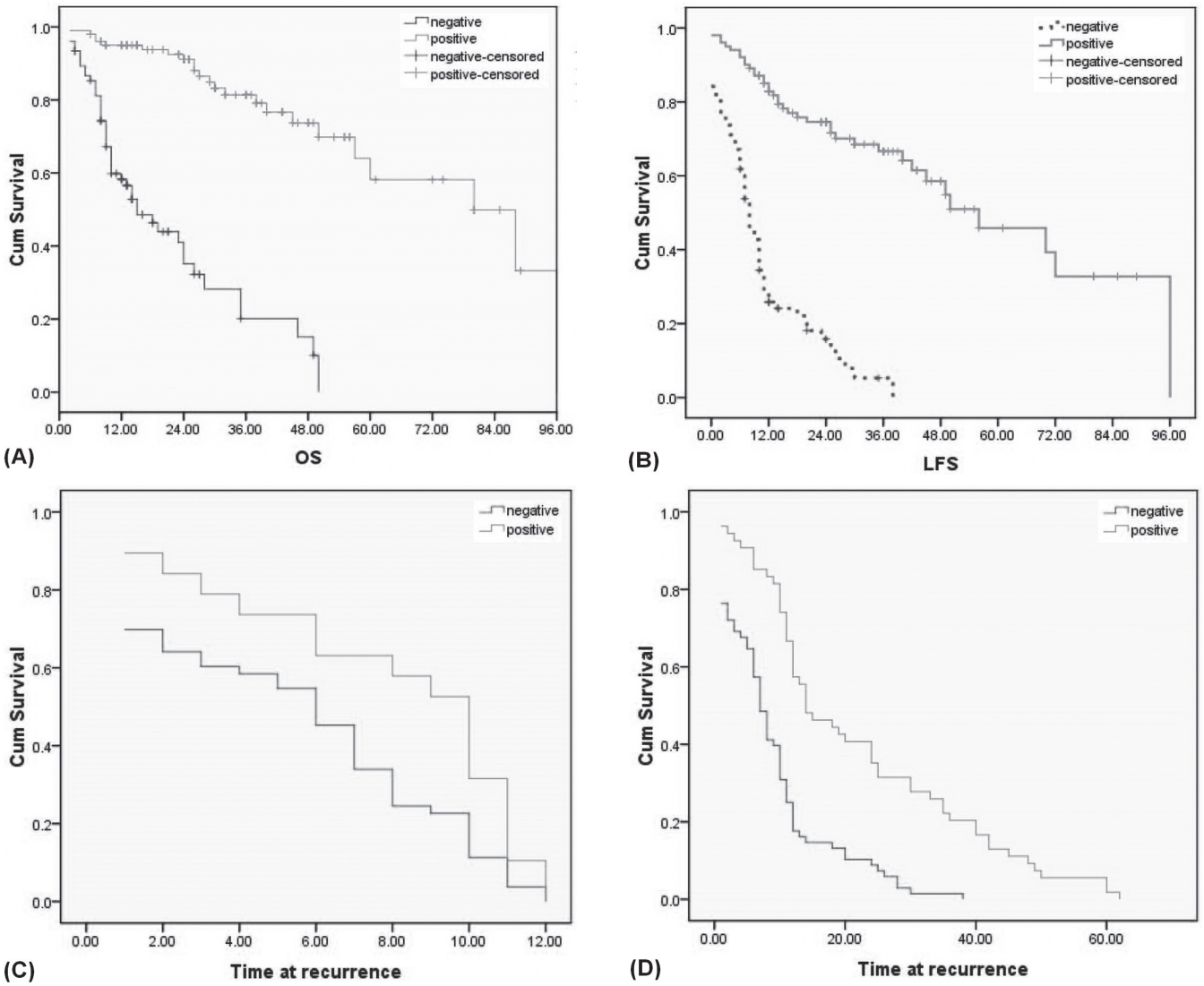

FIGURE 1 Survival and relapse of adult patients with acute myeloid leukemia according to hematogone (HG) status (HG-positive, <1%, and HG-negative, ≥1% by four-colour flow cytometry analysis). (A) Overall survival (OS). (B) Leukemia-free survival (LFS). (C) Recurrences during year 1. (D) Recurrences during years 1–5.

chemotherapy-mediated decline in leukemic cells might promote HG growth and development in bone marrow.

Hematogones can be accurately identified in most bone marrow specimens with the use of optimal antibody combinations in four-color flow cytometry. Hematogones have at least two subtypes, designated HG1 (early-stage population) and HG2 (mid-stage B-cell precursor populations)[11,12]. The number of HGS in bone marrow is highly variable. Higher numbers are more commonly found in children, with even higher numbers being found in regenerating marrow and in some clinical conditions, particularly cytopenias and neoplastic diseases[13]. A general decline in HGS occurs with increasing age and with increasing marrow involvement by neoplastic cells. Because of the high variability of HG numbers in childhood bone marrow, we selectively studied HGS in adult AML patients so as to exclude the influence of age.

Undoubtedly, heredity has a great effect on AML pathogenesis and outcome. Although HGS have already been repeatedly reported to be a sensitive, stable, and independent prognostic factor in AML, the validity of that observation has to be assessed for the various races. In the present work, we divided 177 Chinese adult AML patients who were all initially HG-negative into HG-positive and HG-negative groups after standard chemotherapy. Figure 1 clearly indicates that HGS can also be a valuable prognostic factor in Chinese adult patients with AML. Patients who were HG-positive survived for significantly longer [Figure 1(A,B)] and had a lower relapse rate [Figure 1(C,D)]. We also compared general features, serologic parameters, and phenotypic characteristics including age, peripheral-blood WBCS and blasts, lymphatic antigen co-expression, and abnormal chromosome karyotype in bone marrow cells between HG-positive and HG-negative patients. Figure 3

shows the significantly lower levels of peripheral WBCs, β2MG, and LDH observed in HG-positive patients. Because all those factors potentially lead to poor outcomes[14–17], the lower levels in HG-positive patients further support the idea that HGs could be a valid prognostic factor for Chinese adult patients with AML.

Leukemic cells can be distinguished from normal hematopoietic cells on the basis of morphology, chromosomal or molecular abnormalities, and immunophenotype. With the use of optimal antibodies in flow cytometry, the distinction can almost always be made. After remission, remnants of LSCs were compared in the HG-positive and HG-negative patients. Leukocyte interleukin-3 receptor α (CD123) is a marker of LSCs, and overexpression of CD123 on LSCs is correlated with blast proliferation and poor prognosis[18,19]. Figure 5 suggests that significantly decreased numbers of LSCs and CD34-positive cells were observed in bone marrow from HG-positive AML patients. Table II demonstrates the correlation of survival with some commonly detected factors in such patients. Although other factors such as age, peripheral WBCs, and β2MG were also correlated with survival, the correlation coefficient for HGs with survival was the highest.

It is well known that the response to chemotherapy in AML patients varies with FAB type; some types are much more sensitive to chemotherapy than others. Here, we evaluated whether the various subtypes of AML were significantly associated with distinct HG levels as well as with distinct outcomes. Figure 6 clearly demonstrates that, compared with patients having other types of AML, those having the M3 type of AML, who achieved good responses to chemotherapy as verified by clinical studies, also had significantly higher levels of HGs.

We clearly demonstrated that HG level is a valuable prognostic factor for Chinese adult AML patients; however, to benefit our clinical work, other information was also required. We had to determine whether remission could be achieved with one round of standard chemotherapy, and whether additional chemotherapy could further increase HG levels and thereby improve outcomes. To address that issue, 68 patients were given additional intensified chemotherapy after receiving the initial standard chemotherapy protocol. Bone-marrow samples were collected after the 1st and the 2nd rounds of chemotherapy, and HGs in the samples were evaluated. Figure 6 confirms the effectiveness of the additional intensified chemotherapy. When HGs in bone marrow samples were compared before and after the intensive chemotherapy, a further increase in the HG level was detected in patients who had received 2

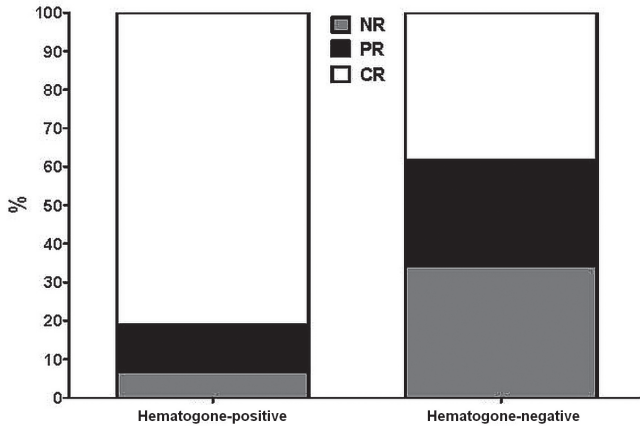

FIGURE 2 Remissions after first chemotherapy, by hematogone status. Remissions were significantly increased in hematogone-positive patients (p = 0.000). NR = no remission; PR = partial remission; CR = complete remission.

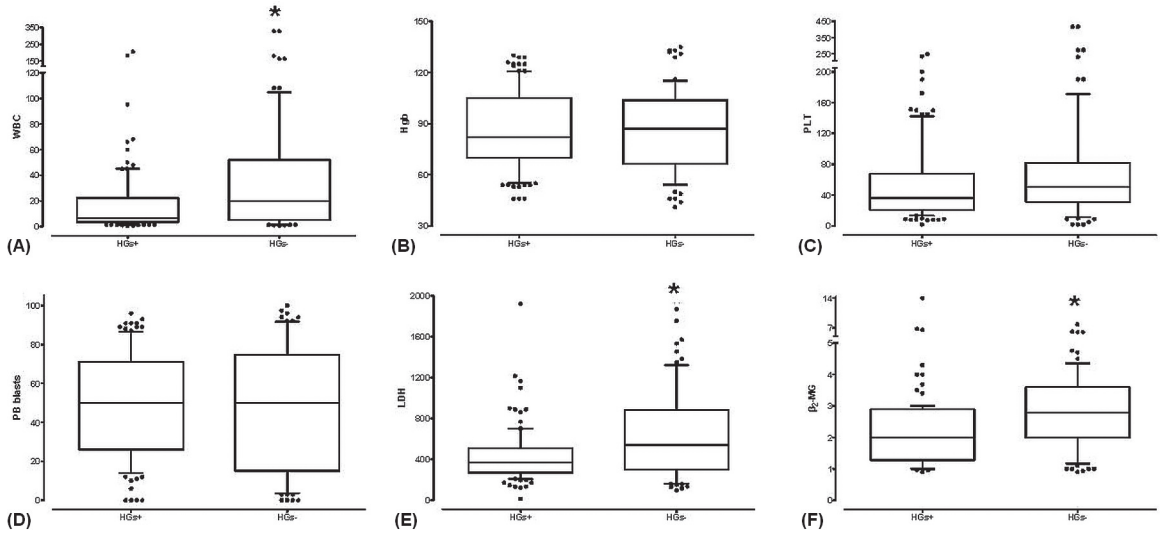

FIGURE 3 Serologic parameters at diagnosis in hematogone-positive (HG+) and -negative (HG–) patients with acute myeloid leukemia after chemotherapy. (A) White blood cells (WBC). (B) Hemoglobin (Hgb). (C) Platelets (PLT). (D) Peripheral blood (PB) blasts. (E) Lactate dehydrogenase (LDH). (F) β_2-microglobulin (β_2-MG). * Statistically significant ($p < 0.05$).

TABLE II Prognostic factors associated with survival in patients with acute myeloid leukemia

Survival parameter and variables examined	Univariate analysis			Multivariate analysis		
	RR	95%CI	*p* Value	RR	95%CI	*p* Value
Overall survival						
Hematogones	0.554	0.455 to 0.675	0.000	0.734	0.597 to 0.902	0.003
Age	1.025	1.011 to 1.040	0.001	1.022	1.006 to 1.037	0.005
Sex	0.799	0.495 to 1.289	0.358			
White blood cells	1.004	1.000 to 1.008	0.040			
Hemoglobin	0.997	0.998 to 1.007	0.612			
Platelets	1.002	0.999 to 1.005	0.238			
Lactate dehydrogenase	1.001	1.000 to 1.002	0.001			
β_2-microglobulin	1.351	1.107 to 1.647	0.003			
PB blasts	0.996	0.987 to 1.004	0.325			
BM blasts	0.992	0.984 to 1.000	0.052	0.991	0.982 to 1.000	0.042
Leukemia stem cells	1.022	1.011 to 1.033	0.000			
Risk stratification						
Better	Reference			Reference		
Intermediate	10.377	4.353 to 24.740	0.000	5.747	2.163 to 15.267	0.000
Poor	13.397	5.393 to 33.278	0.000	6.370	2.264 to 17.926	0.000
Leukemia-free survival						
Hematogones	0.559	0.475 to 0.659	0.000	0.751	0.635 to 0.887	0.001
Age	1.021	1.009 to 1.033	0.000			
Sex	0.605	0.408 to 0.895	0.012			
White blood cells	1.005	1.002 to 1.008	0.000			
Hemoglobin	0.998	0.990 to 1.006	0.663			
Platelets	1.002	0.999 to 1.005	0.205			
Lactate dehydrogenase	1.001	1.001 to 1.002	0.000			
β_2-microglobulin	1.579	1.354 to 1.842	0.000			
PB blasts	1.002	0.995 to 1.009	0.592			
BM blasts	0.997	0.990 to 1.004	0.411			
Leukemia stem cells	1.015	1.007 to 1.024	0.000			
Risk stratification						
Better	Reference			Reference		
Intermediate	8.972	4.531 to 17.764	0.000	5.257	2.452 to 11.273	0.000
Poor	11.474	5.649 to 23.307	0.000	6.393	2.886 to 14.162	0.000

RR = risk ratio; CI = confidence interval; PB = peripheral blood; BM = bone marrow.

rounds of chemotherapy. That observation implies that a round of intensive chemotherapy after a round of standard chemotherapy might help to improve outcomes in adult patients with AML.

We initially demonstrated the effectiveness of HGS as a prognostic factor for Chinese adult patients with AML. Compared with their HG-negative peers, HG-positive patients not only experienced a longer survival time and higher remission rate, but also showed serologic and phenotypic changes such as a lower peripheral WBC count, LDH level, and β2MG level at the time of initial diagnosis, and fewer LSCs and CD34-positive cells in bone marrow after standard chemotherapy. In addition, different types of AML were separately evaluated for their association with HG level. The M3 type of AML is reported to respond well to chemotherapy, and compared with other types of AML, it also demonstrated higher levels of bone marrow HGS. Finally, an additional round of intensive chemotherapy after a round of standard chemotherapy can further increase HGS even when remission is achieved by the initial round of chemotherapy. That observation suggests that intensive chemotherapy could potentially improve outcomes in AML patients.

ACKNOWLEDGMENTS

Our study was partly supported by the National Natural Science Foundation of China (no. 81170472), the Natural Science

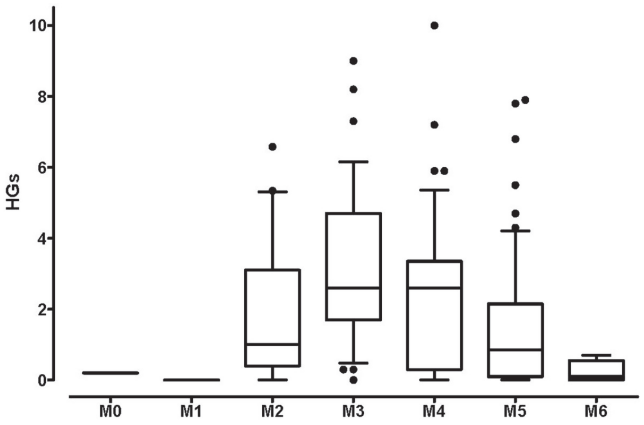

FIGURE 4 Hematogones (HGs) after chemotherapy in various types of acute myeloid leukemia (French–American–British classification before chemotherapy). Too few samples were available to assess statistical differences between the M0 and M1 types. In M2–M6 disease, HG percentages were 1.85% ± 1.91%, 3.20% ± 2.19%, 2.39% ± 2.23%, 1.54% ± 1.89%, and 0.23% ± 0.30% respectively. The highest percentage of HGs was observed in M3 disease.

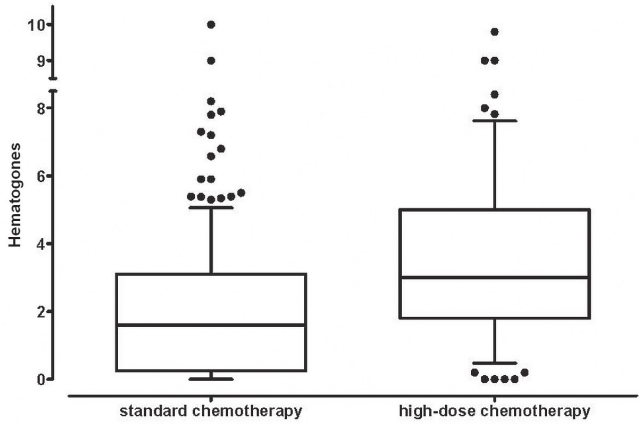

FIGURE 6 Hematogones in bone marrow by flow cytometry after an additional course of extra-high-dose chemotherapy (68 of 177 patients) compared with after standard-dose chemotherapy (all 177 patients).

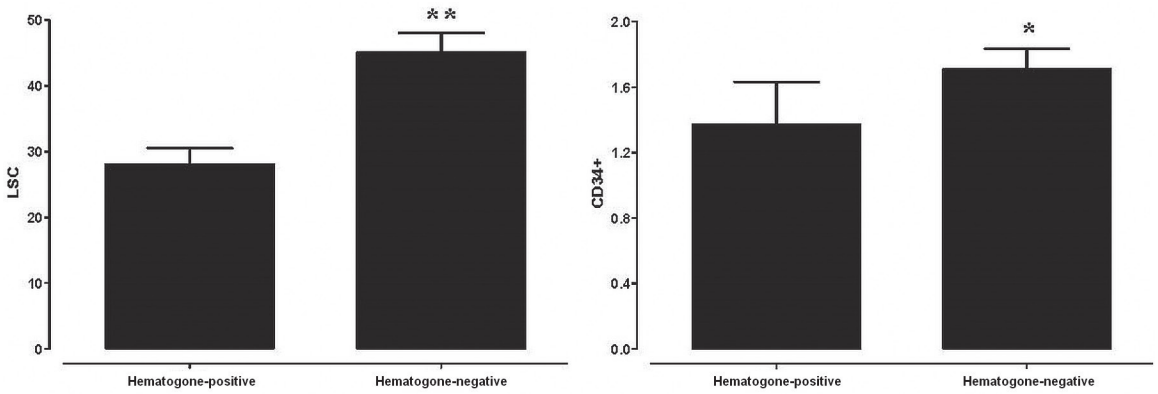

FIGURE 5 Percentage of leukemia stem cells (LSCs) and CD34+ cells detected by flow cytometry in bone marrow from non-M3 acute myeloid leukemia patients, by hematogone status. ** $p < 0.01$; * $p < 0.05$.

Foundation of Tianjin (no. 15JCYBJC24300), the Tianjin Cancer Major Projects Research Plan (no. 12ZCDZSY17900 and 12ZCDZSY18000), and the National Public Health Grand Research Foundation (no. 201202017).

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

*Department of Hematology, General Hospital of Tianjin Medical University, Tianjin, P.R.C.; †Department of Health Statistics, School of Public Health, Tianjin Medical University, Tianjin, P.R.C.

REFERENCES

1. Davis RE, Longacre TA, Cornbleet PJ. Hematogones in the bone marrow of adults. Immunophenotypic features, clinical settings, and differential diagnosis. *Am J Clin Pathol* 1994;102:202–11.
2. McKenna RW, Washington LT, Aquino DB, Picker LJ, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry. *Blood* 2001;98:2498–507.
3. Mlcakova A, Babusikova O. Multiparametric flow cytometry in detection of minimal residual disease in acute lymphoblastic leukemia of early B-cell phenotype. *Neoplasma* 2003;50:416–21.
4. Akyay A, Falay M, Ozturkmen S, *et al.* Hematogones in immune thrombocytopenic purpura: diagnostic implication. *Turk J Pediatr* 2011;53:219–24.
5. Carulli G, Ottaviano V, Guerri V, *et al.* Multiparameter flow cytometry to detect hematogones and to assess B-lymphocyte clonality in bone marrow samples from patients with non-Hodgkin lymphomas. *Hematol Rep* 2014;6:5381.
6. Honebrink T, Dayton V, Burke MJ, *et al.* Impact of bone marrow hematogones on umbilical cord blood transplantation outcomes in patients with acute myeloid leukemia. *Biol Blood Marrow Transplant* 2012;18:930–6.

7. Chantepie SP, Salaun V, Parienti JJ, *et al*. Hematogones: a new prognostic factor for acute myeloblastic leukemia. *Blood* 2011;117:1315–18.
8. Chu SC, Wang TF, Su YC, *et al*. Prognostic significance of flow cytometric residual disease, dysregulated neutrophils/monocytes, and hematogones in adult acute myeloid leukemia in first remission. *Int J Hematol* 2014;99:296–304.
9. Hrusak O, Porwit-MacDonald A. Antigen expression patterns reflecting genotype of acute leukemias. *Leukemia* 2002;16:1233–58.
10. Ribeiro E, Matarraz Sudón S, de Santiago M, *et al*. Maturation-associated immunophenotypic abnormalities in bone marrow B-lymphocytes in myelodysplastic syndromes. *Leuk Res* 2006;30:9–16.
11. Babusikova O, Zeleznikova T, Mlcakova A, Kusenda J, Stevulova L. The knowledge on the 3rd type hematogones could contribute to more precise detection of small numbers of precursor B-acute lymphoblastic leukemia. *Neoplasma* 2005;52:502–9.
12. McKenna RW, Asplund SL, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) and neoplastic lymphoblasts by 4-color flow cytometry. *Leuk Lymphoma* 2004;45:277–85.
13. Braham Jmili N, Nsaibia S, Jacob MC, *et al*. Immunophenotypic analysis of bone marrow B lymphocyte precursors (hematogones) by flow cytometry. *Clin Lab Sci* 2009;22:208–15.
14. Chen X, Xie H, Wood BL, *et al*. Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. *J Clin Oncol* 2015;33:1258–64.
15. Bahl A, Sharma A, Raina V, *et al*. Long-term outcomes for patients with acute myeloid leukemia: a single-center experience from AIIMS, India. *Asia Pac J Clin Oncol* 2015;11:242–52.
16. Estey EH. Acute myeloid leukemia: 2014 update on risk-stratification and management. *Am J Hematol* 2014;89:1063–81.
17. Zheng J, Du W, Yao J, *et al*. Analysis of hematogones in bone marrow from acute myeloid leukaemia cases posttherapy. *Eur J Clin Invest* 2013;43:1140–6.
18. Yalcintepe L, Frankel AE, Hogge DE. Expression of interleukin-3 receptor subunits on defined subpopulations of acute myeloid leukemia blasts predicts the cytotoxicity of diphtheria toxin interleukin-3 fusion protein against malignant progenitors that engraft in immunodeficient mice. *Blood* 2006;108:3530–7.
19. Li LJ, Tao JL, Fu R, *et al*. Increased CD34+CD38−CD123+ cells in myelodysplastic syndrome displaying malignant features similar to those in AML. *Int J Hematol* 2014;100:60–9.