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ORIGINAL PAPER

Outcome of acute myeloid leukemia patients with hyperleukocytosis in Brazil

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Abstract Acute myeloid leukemia (AML) with a high white blood cell (WBC) count at presentation has been associated with an increased early mortality rate, usually secondary to leukostasis. However, the value of the WBC count at which there is a high risk of early death (ED) and the efficiency of supportive treatments remain unclear. In this report, a series of 187 consecutive adult patients with AML in our institution was reviewed. The outcome of 40 patients with WBC above 50×10^9 L^{-1} (hyperleukocytosis) was compared to 147 patients with a leukocyte count lower than 50×10^9 L^{-1}. The group with hyperleukocytosis showed a significantly shorter OS ($P < 0.0001$) and a higher rate of ED ($P = 0.0008$). Even when the data from ED patients were removed from analysis, we still detected a shorter OS in patients with hyperleukocytosis ($P = 0.0049$), which suggests that high WBC number influences long-term survival, and not only ED. We also observed higher lactic dehydrogenase (LDH) and serum creatinine levels in the group of patients with hyperleukocytosis ($P = 0.0003$ and 0.0406, respectively). Besides considering all the patients with ED, we could observe higher levels of lactic dehydrogenase, a serum creatinine and nitrogen urea ($P = 0.0056$, $P = 0.0008$ and $P < 0.0001$, respectively). Pulmonary involvement was more frequent in patients with ED ($P = 0.0277$). In conclusion, hyperleukocytosis confers a poorer prognosis in patients with AML.

Keywords Acute myeloid leukemia · Hyperleukocytosis · Early death

Introduction

Despite the improvement of remission rate and overall survival in acute myeloid leukemia (AML) observed in the last two decades, high rates of early death (ED), usually defined arbitrarily as death occurred in the first week of diagnosis, still represent a challenging therapeutic problem, especially in the context of patients with hyperleukocytosis at presentation. Hyperleukocytosis, conventionally defined as blast count superior to 50–100 $\times 10^9$ L^{-1} in peripheral blood (PB), has been associated with an unfavorable prognosis due to early death and a higher risk of relapse [1, 2]. Although the mechanisms of hyperleukocytosis are poorly understood, it was demonstrated that the malignant blasts are capable of adhering to the vascular endothelium and transmigrate into tissues [3–5], a condition called leukostasis syndrome (LS), which leads to the occlusion of small arteries in the brain, lungs and other organs by aggregates of blast cells and by blast cell thrombi, which causes hemorrhage, respiratory failure and higher relapse rate in the central nervous systems (CNS). The LS is rapidly progressive and fatal in the majority of patients [6]. The clinical picture of this condition derives from involvement of any organ [7, 8]. Causes of death include intracerebral hemorrhage and respiratory and renal failure. Five to 18%

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of adults with AML present hyperleukocytosis, and it is considered a risk factor to ED in 20–40% of patients [8].

Studies on the impact of leukapheresis on overall mortality and early death are scant, but they do not show that this therapeutic approach contributes to improve survival [9, 10].

Geographic differences have been reported regarding AML clinical and laboratorial features. In Brazil, it has been shown that a higher frequency of AML with t(15;17), higher WBC counts at diagnosis, elevated ED rates and poorer responses to treatment [11–13]. Nevertheless, the impact of hyperleukocytosis as prognostic factor on AML in this population has not been established. Our aim in this retrospective study is to evaluate the impact of the leukocyte count at presentation on outcome of newly diagnosed *de novo* or secondary to myelodisplastic syndrome in Brazilian patients with AML [1, 2, 14].

Materials and methods

Patients' characteristics

We reviewed the data of 187 consecutive patients with AML treated at our institution (Hospital das Clínicas de Ribeirão Preto da Universidade de São Paulo-USP) between January 1998 and June 2008 (Table 1). We excluded from the analysis patients with acute promyelocytic leukemia and blastic crisis in chronic myelocytic leukemia. Pediatric patients (age under 15) were also excluded. AML *de novo* was classified based on blast cell morphology, cytochemistry and immunophenotypical analysis according to the French-American-British (FAB) classification [15]. AML with myelodysplasia-related changes, AML therapy-related and acute leukemia of ambiguous lineage were categorized separately [16–18]. We did not consider the karyotype results because cytogenetic analysis was performed only in the last few patients. The initial white blood cell count, age at diagnosis, gender, serum creatinine, blood urea nitrogen and LDH were recorded. We also evaluated symptoms and clinical parameters at presentation such as presence of fever or hepatosplenomegaly on physical examination, respiratory distress (hypoxemia, tachypnea) and CNS involvement, defined as confusion, lethargy, stupor, blurred vision, coma, papilledema, retinal vein distension or retinal hemorrhages.

The patients were classified into two groups according to their white blood cell (WBC) count at diagnosis: with WBC $\leq 50 \times 10^9$ L^{-1} with WBC $> 50 \times 10^9$ L^{-1}. We also compared the patients' characteristics who died within the first 7 days (defined as ED), and the patients who survived further on this period.

Table 1 Characteristics of patients with acute myeloid leukemia

Characteristics	Number of patients, $n = 187$
Median age	51 (15–86)
Gender (male/female)	97/90
Median WBC ($\times 10^9$ L^{-1}) count	10.2 (0.3–420)
Median LDH (U/L)	728 (121–5,877)
Median urea (mg/dL)	32 (9–226)
Median creatinine (mg/dL)	1.0 (0.4–4.5)
FAB classification	
M0	14
M1	13
M2	48
M4	29
M5	9
M6	11
M7	4
AML with myelodysplasia-related changes	28
AML therapy-related	1
Acute leukemia of ambiguous lineage	5
Not categorized	25
Fever (yes/no)[a]	20/36
Pulmonary involvement (yes/no)[a]	12/44
Liver/spleen enlargement (yes/no)	65/122
CNS involvement (yes/no)	58/129
Chemotherapy (at least 2 days; yes/no)	172/15
Median overall survival (days)	115 (0–2,627)

WBC indicates white blood cell, *LDH* lactic dehydrogenase, *FAB* French-American-British, *AML* acute myeloid leukemia, *CNS* central nervous system

[a] This variable was analyzed in only 56 patients

This study was approved by our institution Ethics Committee (number 8736/2008).

Flow cytometric analysis

The leukemic cells were resuspended in phosphate-buffered saline (PBS) before labeling. A panel of mouse antibodies (MoAbs) directly conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) was used. The leukemic blasts were identified by gating the typical formation in the forward/sideward scatter projection, with blasts, residual lymphocytes and monocytes included. Appropriate isotypic controls were used. Results were expressed as the percentage of positive events in the defined gate compared with the isotype control by using CellQuest Software (Becton–Dickinson). If the percentage of positive events was higher than 20%, the cell population was considered positive for the marker [19].

Chemotherapy

The absolute value of blast count at presentation was not considered when choosing the chemotherapy schedule. One hundred and seventy-two (91.98%) patients underwent to at least 2 days of chemotherapy. Patients under 60 years were submitted to a chemotherapy which consisted of the association of cytarabine with an anthracycline, alone or preceded by few days of high doses of hydroxyurea. Patients over 60 years were treated daily with low doses of cytarabine (20 mg/m^2), for 21 days, followed by a period of 21 days without chemotherapy. The number of cycles varied according to the patient response and clinical status.

Leukapheresis

Patients with AML were eligible to therapeutic leukapheresis when they presented at the outset with symptomatic hyperleukocytosis and WBC $> 100 \times 10^9$ L^{-1}, except when the patient showed signs of circulatory instability. The decision to perform leukapheresis is taken based on the discretion of the assisting hematologist.

The procedures of leukapheresis were performed with a continuous-flow blood cell separator (COBE Spectra PMN manual program). The objective was to process 2 blood volemias, in approximately 2 h. For anticoagulation, acid citrate dextrose Formula A was added at a ratio of 1 :14. Hydroxyethyl starch was not employed. Blood cell counts were done before and immediately after the procedure. The leukapheresis was continued on a daily basis until leukemic blast count was inferior to 100×10^9 L^{-1}. Red blood cell transfusion was avoided during the period of hyperleukocytosis in order to prevent increment of blood viscosity.

Statistical methods

Descriptive analyses were carried out for all variables, and their means were checked by Shapiro–Wilk test to evaluate the Gaussian distribution. Comparison of categorical variables (clinical features or events) was performed using Fisher's exact test. Continuous variables were compared using unpaired non-parametric test (Mann–Whitney). Overall survival (OS), defined as the time elapsed from the date of diagnosis to the date of death, was estimated by Kaplan–Meier method followed by the log-rank test to check the equality survivor functions among groups. Data with non-Gaussian distribution were shown as median and range. For the whole analysis, statistical significance was assumed if $P < 0.05$.

Results

Patients' characteristics

One hundred and eighty-seven patients with AML were eligible for this study. Their characteristics are shown in Table 1. There were 97 men and 90 women. The median age was 51 years (range 15–86). Eighteen from the 187 patients (9.63%) died within the first 7 days of diagnosis. Out of the 40 patients with hyperleukocytosis (21.39%), 10 (25%) died within 7 days, whereas in the group with leukocyte count inferior to 50×10^9/L, 8 (5.44%) from the 147 died within this period ($P = 0.0008$; Table 2). There was no difference in frequency of hyperleukocytosis when we divided the population of patients into 2 groups according to age, one under and the other over 60 ($P = 0.7278$).

Analysis of the factors associated with hyperleukocytosis

Median age was 50.5 years (15–86) and 51.0 years (17–78) in the groups with and without hyperleukocytosis, respectively. Similarly, the gender distribution was similar between the 2 groups (Table 2). However, LDH and creatinine levels were higher in the group of patients with hyperleukocytosis ($P = 0.0003$ and 0.0406, respectively). Besides, the presence of liver/spleen enlargement and CNS involvement were more frequent in the group with hyperleukocytosis ($P = 0.0259$ and 0.0356, respectively). The incidence of pulmonary involvement, analyzed only in 56 patients, was not different ($P = 0.3483$). Serum urea and presence of fever were not associated with WBC count.

Analyses of the factors associated with early death

Higher WBC count was strongly associated with death within the first 7 days ($P < 0.0001$; Table 3). Other variables identified that were associated with higher rate of ED were elevated levels of lactic dehydrogenase (LDH; 1,181 U/L vs 672.3, $P = 0.0056$), serum creatinine and nitrogen urea (1.85 mg/dL vs 0.90 mg/dL, $P = 0.0008$) and (63.5 mg/dL vs 31.0 mg/dL, $P < 0.0001$), respectively. Pulmonary involvement was more frequent in patients with ED ($P = 0.0277$). Other clinical parameters were not different between the two groups (Table 3).

Overall survival

The OS of the total population was 115 days (range 0–2,627), whereas the OS of patients with and without hyperleukocytosis was 30 (range 0–1,425) and 150 (range 1–2,627) days ($P < 0.0001$), respectively (Fig. 1). When

Table 2 Comparison of parameters according to white blood count (WBC)

	WBC $\leq 50 \times 10^9$ L^{-1}	WBC $> 50 \times 10^9$ L^{-1}	*P*-Value
Number of patients	147	40	
Median age	50.5 (15–86)	51 (17–78)	0.6969
Gender (male/female)	81/66	16/24	0.1089
Early death[a]	8	10	**0.0008**
Median LDH (U/L)	643 (121–3,624)	1142 (293–5,877)	**0.0003**
Median urea (mg/dL)	32 (9–226)	33.65 (12–133)	0.6565
Median creatinine (mg/dL)	0.9 (0.4–4.5)	1.2 (0.5–4.1)	**0.0406**
Fever (yes/no)[b]	1/32	4/4	0.4369
Pulmonary involvement (yes/no)[b]	9/39	3/5	0.3483
Liver/spleen enlargement (yes/no)	45/102	20/20	**0.0259**
CNS involvement (yes/no)	40/107	18/22	**0.0356**
Chemotherapy (yes/no)	138/9	34/6	0.0952
Median overall survival (days)	150 (1–2,627)	30 (0–1,425)	**<0.0001**

Significant differences are in bold

WBC indicates white blood cell, *LDH* indicates lactic dehydrogenase, *CNS* central nervous system

[a] Early death was defined as the death that occurred within the first 7 days of diagnosis

[b] This variable was analyzed in only 56 patients

the data of ED patients were removed from analysis, the OS remained significantly shorter in patients with hyperleukocytosis ($P = 0.0049$).

Discussion

The hyperleukocytosis impact on the prognosis in AML is not completely known. About 10% of our patients had more than 100×10^9 L^{-1} at diagnosis, which is similar to the reported in developed countries [1, 20, 21]. In this study, we showed that hyperleukocytosis (here considered WBC $> 50 \times 10^9$ L^{-1}) is an important predictive factor of ED in AML, even considering the finding that OS at our institution is poorer than previously reported by others [1, 2]. This poor outcome can probably be explained by the inferior support infrastructure available for our patients. Another explanation could be the fact that most of our patients live in poor sanitary conditions and relatively distant from the hospital where they are treated. Some investigators have reported an association between low socioeconomic condition and poor survival in patients with AML [22]. It can be assumed that the population in higher socioeconomic ranks live in urban areas and, consequently, have easier access to a better health care. We cannot rule out the possibility of the influence of ethnicity on prognosis in AML, as it has been suggested by others [23].

Among our 40 patients with hyperleukocytosis, 10 (25%) died within the first 7 days with a suggestive clinical picture of leukostasis. Our finding that hyperleukocytosis is associated with higher rate of early mortality in patients with AML is in accordance with findings reported by previous authors either in a pediatric group of patients [7, 24] and in adults [1, 14]. When we established an arbitrary cutoff as low as 10,000/μL, we could demonstrate its impact on OS ($P = 0.0094$; data not shown), which suggests a close correlation between WBC and OS rather than a pre-established cutoff value.

Furthermore, removing the data of all ED patients from the analysis, the OS remained significantly shorter in the group with hyperleukocytosis ($P = 0.0049$). This fact indicates that high leukocyte count is a biological marker of somber prognosis irrespective of early complications as leukostasis, which agrees with observations recently reported by Marbello et al. [14], but not with others [2].

Some investigators obtained results suggesting that supportive treatment does not seem to decrease early mortality [1, 2]. Ten from 18 patients (55.5%) with WBC count $> 100 \times 10^9$ L^{-1} and symptoms and/or signs of leukostasis were treated with hydroxyurea associated with leukapheresis. This group of patients did not have better survival rates compared to the group of 8 patients with WBC $> 100 \times 10^9$ L^{-1} treated with hydroxyurea alone (data not shown). This finding suggests that supportive therapy with leukapheresis does not influence on outcome of AML patients, as shown by others [8, 9]. On the other hand, some investigators suggested previously a better outcome in patients with hyperleukocytosis submitted to leukapheresis [25, 26].

This retrospective study was performed by analyzing data obtained from patients in a single institution. Since we do not have the cytogenetics analysis for most of the

Table 3 Comparison of parameters according to survival

	Survival $\leq$ 7 days	Survival $>$ 7 days	*P*-Value
Number of patients	18	169	
Median age (years)	54 (16–81)	50.5(15–86)	0.3691
Gender (male/female)	11/7	86/83	0.4642
Median WBC ($\times 10^9$ L^{-1}) count	71.15 (1.2–299)	7.7 (0.3–420)	**<0.0001**
Median LDH (U/L)	1181(273–5,209)	672.3 (121–5,877)	**0.0056**
Median urea (mg/dL)	63.5 (17–164)	31 (9–226)	**<0.0001**
Median creatinine (mg/dL)	1.85 (0.5–4.5)	0.9 (0.4–4.1)	**0.0008**
Fever (yes/no)[a]	3/1	17/35	0.1249
Pulmonary involvement (yes/no)[a]	3/1	9/43	**0.0277**
Liver/spleen enlargement (yes/no)	10/8	55/114	0.0679
CNS involvement (yes/no)	8/10	50/119	0.2823
Chemotherapy (at least 2 days; yes/no)	7/11	166/3	**<0.0001**

Significant differences are in bold

WBC indicates white blood cell, *LDH* lactic dehydrogenase, *CNS* central nervous system

[a] This variable was analyzed in only 56 patients

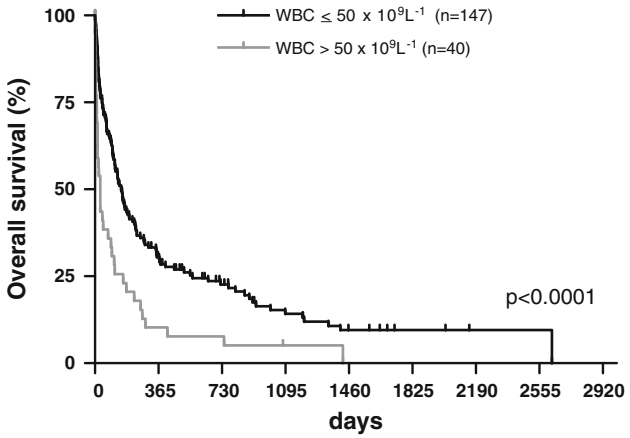

Fig. 1 Comparison of overall survival between the group of patients with WBC $\leq 50 \times 10^9$ L^{-1} and with WBC $> 50 \times 10^9$ L^{-1}

patients, we could not evaluate the impact of this important prognostic factor on survival, relapse rate and ED.

In summary, more than 20% of patients with AML have WBC count above 50,000/μL at presentation. These patients are defined as having hyperleukocytosis, which confers a higher ED risk for them than for the patients with inferior number of leukocyte cells. Other biological variables were associated with elevated WBC count and poorer prognosis, such as hepatosplenomegaly, CNS involvement, higher levels of lactic dehydrogenase and impaired renal function. Considering all these findings, we believe that it is important to improve risk stratification for patients with AML in order to develop more accurate treatment strategies.

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