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CD20 expression in B-cell precursor acute lymphoblastic leukemia is common in Mexican patients and lacks a prognostic value

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Classification of acute lymphoblastic leukemia (ALL) by flow cytometric immunophenotyping characterizes the disease and delineates potential therapeutic intervention. We retrospectively analyzed CD20 expression in 143 patients with newly diagnosed precursor B-cell ALL. CD20 was observed in 61% of patients at diagnosis. There was no correlation between CD20 expression and age, white blood cell count, or cytogenetic abnormalities. Despite the fact that CD20-positive ALL patients had a tendency toward a worse outcome, there was no significant difference between patients with and without CD20 expression in 3-year overall survival 65 vs. 82% ($P = 0.14$), and cumulative incidence of relapse 36 vs. 18% ($P = 0.3$) in pediatric patients and 51 vs. 53% ($P = 0.31$) and 35 vs. 38% ($P = 0.6$) in adults, respectively. In conclusion, CD20 expression appears to be more common in Mexican patients with newly diagnosed precursor B-cell ALL higher than in Caucasian populations and lacks prognostic value.

Keywords: CD20, Acute lymphoblastic leukemia, Prognostic factor

Introduction

Improvement in the outcome of treatment of acute lymphoblastic leukemia (ALL) has been achieved; the most significant progress has been obtained by individualized therapy based on prognostic factors and treatment response. Age is one of the most significant factors influencing outcome, related to differences in the biology of the disease and chemotherapy tolerance.[1,2] The subclassification of ALL by flow cytometric (FC) immunophenotyping characterizes the disease and also delineates potential therapeutic interventions by detecting surface antigens such as CD19, CD20, CD22, CD33, and CD52, which can be targeted by specific monoclonal antibodies; most experience is available for anti-CD20.[3,4] The CD20 molecule is a B-lineage-specific antigen expressed on both normal and malignant cells during nearly all stages of B-cell differentiation. CD20 expression ranges from 35 to 48% in precursor B-cell ALL[5–10] compared with 80 to 90% in mature B-cell or Burkitt-type leukemia/lymphoma.[11] In addition, there are pediatric data suggesting that CD20 expression is upregulated during induction chemotherapy, even in cases deemed CD20-negative at baseline.[12] The prognostic relevance of CD20 expression in B-cell ALL has been investigated with conflicting results: both better[5,6] and worse[7–10] impact on treatment outcomes have been described. The aim of the present study was to analyze CD20 expression and determine its prognostic significance in children and adults with newly diagnosed precursor B-cell ALL.

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Materials and methods

Patients with newly diagnosed precursor B-cell ALL treated at the Hematology Service of the Hospital Universitario, 'Dr. José E. González' in Monterrey and in the Centro de Hematología y Medicina Interna, Clínica Ruiz de Puebla from 2005 to 2010 were included; they were required to have both FC phenotype at diagnosis and minimal residue disease

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(MRD) analysis by FC at the end of the induction to remission treatment. Medical records of each patient were reviewed and data on age, sex, white blood cells count, cytogenetic, immunophenotype, and MRD status were retrieved.

Immunophenotyping was performed at each center according to standard recommendations.[3,13] Four-color FC immunophenotypic analysis of bone marrow or peripheral blood at diagnosis and quality control evaluations were performed collecting at least 20 000 cellular events, whereas for MRD measurements 500 000 events were acquired. Cell acquisition was performed with a FACSCalibur cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) using the CellQuest™ software (BD Biosciences, San Jose, CA, USA). Leukemic cells were identified using an immunological gate which included all the CD19+ cells. MRD was defined as an accumulation of at least 10 clustered events displaying leukemia-associated immunophenotypic characteristics. CD20 expression of samples was estimated by assessing the proportion of leukemic cells positive for the antigen with a cut-off of more than or equal to 20%. We defined patients as having positive MRD if FC analysis at the end of the induction to remission was at least 0.01% and negative MRD if it was lower. In all the cases in which MRD was detected comparison was made between MRD sample and diagnostic specimens.

Treatment

Pediatric patients were assigned to standard risk or poor risk protocols based on age, white blood cells count, and cytogenetics. All adult patients were initially treated with standard induction to remission treatment consisting of 28 days glucocorticoids, four weekly vincristine, and doxorubicin or daunorubicin doses, and L-asparaginase as well as triple intrathecal chemotherapy, as previously described.[14] Philadelphia chromosome (Ph1)-positive patients received tyrosine kinase inhibitors in addition to chemotherapy. Anti-CD20 antibodies were not used in any patient.

Statistical methods

SPSS package version 17 (IBM Software Group, Chicago, IL, USA) was used for data analysis. Chi-squared and/or Fisher's exact test were used to compare proportion. Cumulative incidence was used to estimate relapse (CIR) and overall survival (OS) analysis were performed using Kaplan–Meier plots with differences analyzed by the log-rank test.

Results

A total of 143 patients with diagnosis of precursor B-cell ALL were included. The median age of the group overall was 11 (range 1–71) years: 65% were younger than 16 years and 7% were 60 years or older. In 15 patients (10.4%), the Ph1 was found either by karyotype or polymerase chain reaction. CD20 positivity, defined as the expression of CD20 in at least 20% of leukemic blast cells, was observed in 87 of the 143 (61%) patients at diagnosis. There was no significant correlation between CD20 expression and age, white blood cell count, cytogenetic abnormalities, or incidence of Ph1 chromosome. There was a significant higher incidence of CD20 expression in males younger than 16 years, $P = 0.003$ (Table 1).

Of 93 pediatric patients, median age 7 (range 1–15) years, 58 (62%) expressed CD20. There was no association between CD20 expression and leukocyte count or cytogenetics. Median follow-up was 26 (range 1–142) months. Three-year OS was 65% in CD20-positive vs. 82% in the CD20-negative group, ($P = 0.14$); 3-year CIR (35 vs. 18%, $P = 0.3$), respectively, (Fig. 1). In 50 adults, after a median follow-up of 33 (range 2–135) months, median OS, 3-year OS, and CIR between groups with ($n = 29$) and without CD20 ($n = 21$) expression were similar, 41 months vs. not reached ($P = 0.1$), 51 vs. 53% ($P = 0.31$), and 35 vs. 38% ($P = 0.6$), respectively (Fig. 2).

Fifty six (39%) of the 143 patients had detectable leukemic blasts in MRD analysis at the end of the induction to remission treatment. We found no significant difference in CD20 expression at diagnosis and MRD status at the end of induction remission. Of

Table 1 Salient features according to CD20 expression in 143 patients with precursor B-cell ALL

	<16 years			≥16 years		
	CD20+	CD20−	*P* value	CD20+	CD20−	*P* value
Patients	58	35		25	21	
Age median	8	6	0.15	30	37	0.23
Range	1–15	1–15				
Males (%)	39 (67)	12	0.003	15	11	0.7
WBCx109/l median	8.3	9.4	0.97	26	16	0.34
Range	0.60–100	1–61		1.8–400	1–230	
Philadelphia chromosome positive	4	0	0.13	6	4	0.7
Negative MRD (%)	33 (39)	21 (61)	0.83	14	17	0.11
Positive MRD	25 (64)	14 (36)		11	7	

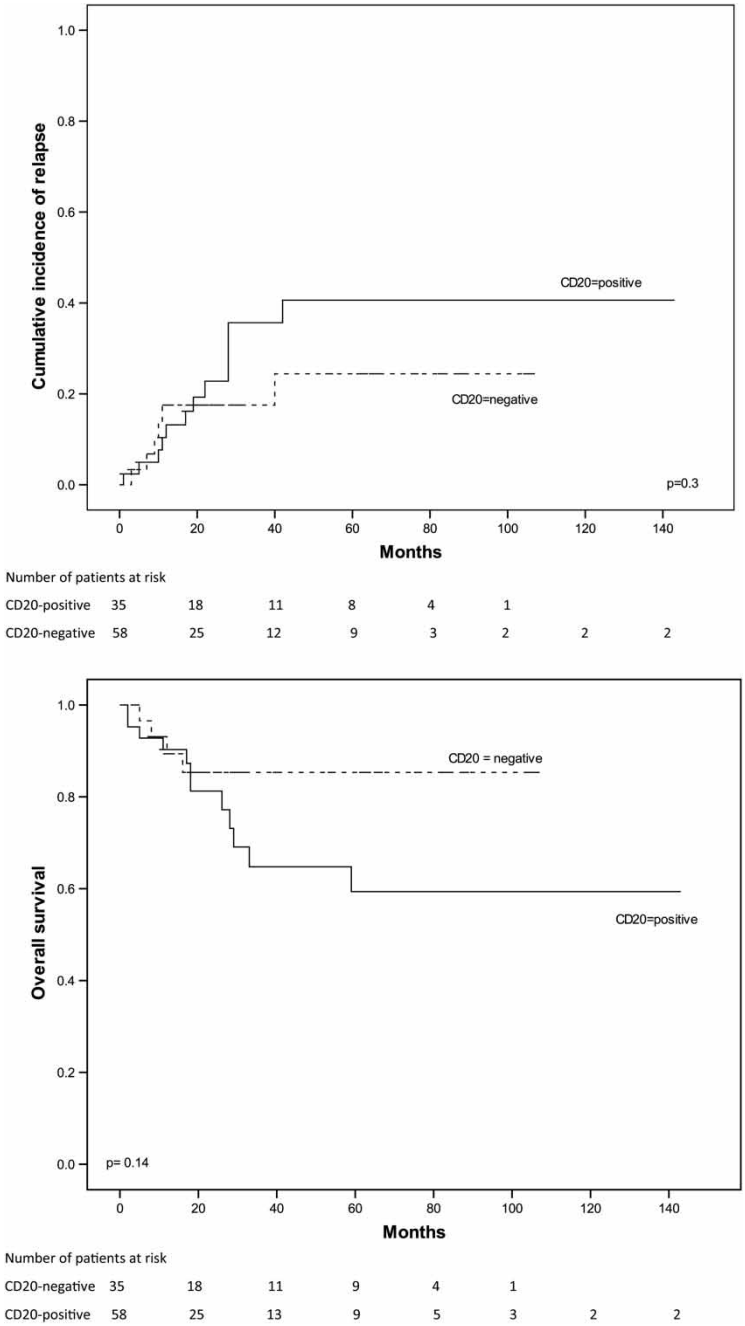

Figure 1 CIR and OS according to CD20 expression in children.

the 87 patients whose leukemic blasts expressed CD20 at diagnosis, 38 (44%) were MRD-positive, whereas 49 (56%) were MRD-negative ($P = 0.21$). Thirty five (92%) of the 38 cases with positive MRD retained CD20-positivity, whereas the leukemic blasts of 3 (8%) patients downregulated the expression of CD20. Of the 56 patients whose leukemic blasts did not express CD20 at diagnosis, 18 (32%) were MRD-positive and 38 (68%) MRD-negative. Thirteen (72%) of the 18 patients with MRD positive remained CD20 negative, whereas 5 (28%) patients whose leukemic blasts initially did not express CD20 upregulated the expression of the antigen showing more than 20% of CD20 expression at MRD analysis. Accordingly, we found that eight patients had a CD20 expression shift, three from CD20-positive at diagnosis to CD20-negative at the end of remission induction, and five patients from CD20-negative at diagnosis to CD20-positive in MRD analysis at the same endpoint.

Discussion

Immunophenotypic classification of ALL has great importance regarding disease characterization, prognosis, and design of therapy. CD20, an important differentiation-related surface antigen of B-cells, is commonly used as a marker in leukemia phenotyping as well as for assessment of MRD in precursor B-cell ALL. We found CD20 expression in 61% of the

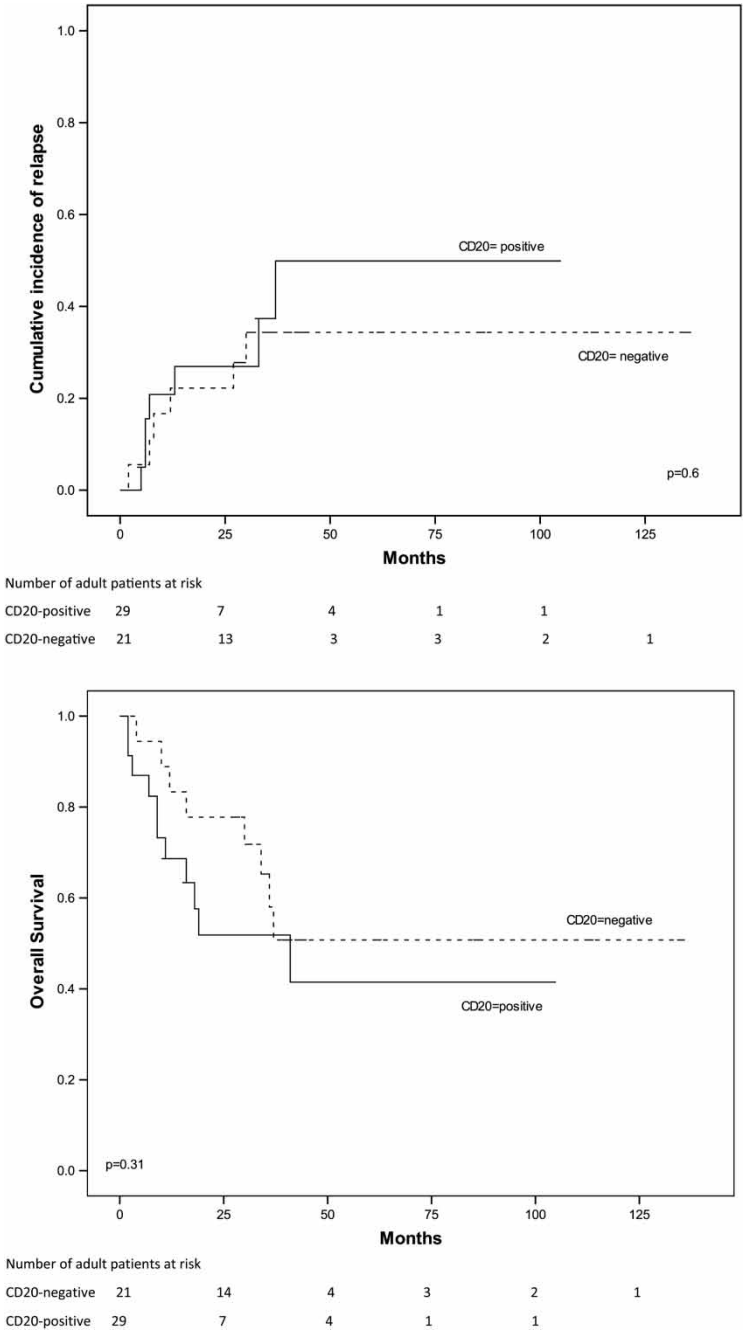

Figure 2 CIR and OS according to CD20 expression in adults.

patients with diagnosis of precursor B-cell ALL, this figure being significantly higher than that previously reported on Caucasian populations: 35–48%.[5–10]

The prognostic relevance of CD20 expression in childhood precursor B-cell ALL has been investigated, with conflicting results. CD20 expression was evaluated in 1231 children treated with risk-adapted protocols in the Pediatric Oncology Group (POG). Absolute CD20 expression was independently associated with a significantly inferior event-free survival irrespective of other known prognostic factors.[9] Jeha and colleagues retrospectively studied the influence of CD20 expression on outcome in 359 children with *de novo* precursor B-cell ALL treated on sequential St Jude Total Therapy protocols (St. Jude Children's Research Hospital, Memphis, TN, USA). The overall incidence of CD20 expression was 48%. In contrast to the POG experience, CD20 expression was associated with a slightly more favorable prognosis. It was postulated that the results could be accounted by differences in the intensity of chemotherapy between the POG and St Jude regimens.[5] We found that there was a slightly, but not significantly less OS in CD20-positive B-cell ALL in children. Similarly, somewhat more frequent relapses, but this was not statistically significant.

In adolescents and adults, CD20 expression was evaluated in 253 patients with *de novo* precursor

B-cell ALL treated in the pre-rituximab era with one of two sequential chemotherapy regimens of increasing intensity at MD Anderson. Forty-seven percent of the cases were CD20-positive. Complete remission rates were similar within the regimens regardless of CD20 status. However, CD20 expression was associated with a higher relapse rate.[7] Another retrospective analysis of CD20 expression in 143 adolescents and adults with the novo precursor B-cell ALL treated with the pediatric-inspired Group for Research in Adult Acute Lymphoblastic Leukemia (GRALL)-2003 regimen identified a higher cumulative incidence of relapse in the CD20-positive subset, although this did not translate in to a difference in disease-free survival.[10] In our analysis, CD20 expression was more common in males; similar to previous studies, there was no correlation between CD20 expression and other well-known prognostic factors. Furthermore, despite the fact that CD20-positive ALL patients had a tendency toward a worse outcome, the difference in OS or cumulative incidence of relapse was not statistically different than that observed in CD20-negative ALL patients.

Changes in the expression of surface antigens on lymphoblasts during the induction phase of chemotherapy have been previously shown;[12,15] these findings have been attributed to glucocorticoid effects.[16,17] In the present study, we observed changes in the CD20 antigen expression in residual leukemic blast cells in some cases. Five of 18 patients with evaluable residual blasts and CD20-negative status at diagnosis showed upregulation of the expression of the antigen at MRD analysis. Two phase II nonrandomized clinical trials suggest that the addition of rituximab to chemotherapy regimens for adolescents and adults with CD20-positive precursor B-cell ALL may improve the outcome.[18,19] Based on the high proportion of the ALL patients included in this study expressing the CD20 antigen, a clinical trial with anti-CD20 antibodies combined with chemotherapy could be considered in our population.

Limitations to our study are a relatively small sample size, short follow-up as well as a selection criterion required to have both FC phenotype at diagnosis and MRD analysis at the end of the induction to remission treatment.

In conclusion, CD20 expression appears to be more common in Mexican patients with newly diagnosed precursor B-cell ALL than in Caucasians, and detectable after therapy in some patients initially being CD20-negative. In addition, CD20 expression had no impact on either OS or CIR.

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