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Prognostic relevance of CD20 in adult B-cell precursor acute lymphoblastic leukemia

We read with great interest the recent article by Maury *et al.* regarding the influence of CD20 expression on prognosis in adult B-cell precursor acute lymphoblastic leukemia (BCP-ALL).[1] This report described their experience with 143 Philadelphia chromosome negative adult BCP-ALL patients treated with GRAALL-2003, a pediatric-like protocol with high dosage of L-asparaginase.[2] Their cohort included 46 (32%) cases with at least 20% of cells expressing CD20. Univariate analysis of CD20 positivity showed no statistically significant effect on overall survival (OS), cumulative incidence of relapse (CIR), and event-free survival (EFS). However, the authors found that CD20 positivity was associated with higher CIR and shorter EFS among the subgroup of patients with high leukocyte count (>30×10^9/L); they also found CD20 expression correlated with higher CIR e in multivariate analysis of the cohort.

We conducted a similar study analyzing the impact of CD20 expression in BCP-ALL. We evaluated 119 patients diagnosed with *de novo* BCP-ALL between the ages of 18 and 60 (median 40 years) at our institute. There were 49 (41%) patients with at least 20% expression of CD20. All patients were initially treated with a modified pediatric protocol including L-asparaginase similar to the one used in the GRAALL-2003 study, as previously described.[3,4] Philadelphia chromosome positive patients received tyrosine kinase inhibitors in addition to this chemotherapy. In total, 32 patients received allogeneic stem cell transplants; these included 14 (29%) of the CD20-positive cases and 18 (26%) of the CD20-negative cases (P=0.83). CD20 positivity was associated with low platelet counts (P=0.004) but not with any other characteristics at diagnosis, including age (median 40.1 years *vs*. 40.0 years) and expression of CD19, CD13, and CD33.

In univariate analysis using the log-rank test, we found no effect of CD20 expression on OS, CIR, or EFS (P=0.18, P=0.40, and P=0.15, respectively). Leukocyte count greater than 30×10^9/L was associated with poorer OS (median 19.9 months *vs*. not reached, P<0.001), CIR (48% *vs*. 26% at two years, P=0.01) and EFS (median 10.6 months *vs*. not reached, P<0.001). Philadelphia chromosome positivity correlated with poorer OS (median 40.8 months *vs.* not reached, P=0.01), CIR (48% *vs.* 23% at two years, P=0.04), and EFS (median 19.3 months *vs*. not reached, P=0.004). Age over 40 had a significant adverse impact on OS (median 80.6 months *vs.* not reached, P=0.05) but not CIR (P=0.41) or EFS (P=0.10). Allogeneic stem cell transplants were associated with shorter OS (median 40.6 months *vs*. 83.0 months, P=0.02), but had no significant difference on cumulative incidence of relapse (P=0.46) or EFS (P=0.10).

Multivariate analysis with Cox's proportional hazards regression was performed on CD20 as well as the four factors identified as significant above: high leukocyte count, Philadelphia chromosome positivity, older age, and allogeneic stem cell transplant. Only high leukocyte count was an independent predictor of an adverse outcome for OS (HR=2.9 [95% CI 1.4-5.8], P=0.003), CIR (HR=2.7 [95% CI 1.1-6.8], P=0.03) and EFS (HR=2.8 [95% CI 1.5-5.22], P=0.002). CD20 positivity did not significantly influence OS (P=0.11) or CIR (P=0.18), and had a trend towards a favorable EFS (HR=0.6 [95% CI 0.3-1.1], P=0.07; Table 1).

Based on the findings by Maury *et al.*, we also performed subgroup analysis of CD20 expression in the low and high leukocyte count groups. Like them, we found no effect of CD20 expression on outcome in the low leukocyte count subgroup (OS: P=0.48, CIR: P=0.35, EFS: P=0.34). However, in contrast to their findings, we found no association between CD20 positivity and adverse outcome among the high leukocyte count subgroup (OS: median 40.2 months *vs.* 12.4 months, P=0.17, Figure 1A; CIR: 47% *vs*. 52% at two years, P=0.63, Figure 1B; EFS: median 33.1 months *vs*. 9.6 months, P=0.18, Figure 1C).

According to the analysis of Maury *et al.*, we then excluded the 14 Philadelphia chromosome positive patients in this high leukocyte count subgroup. Among the 14 remaining patients, there were 5 positive for CD20. All achieved complete remission (CR) on first induction, 2 later relapsed, and one died of relapsed disease. Of the 9 CD20-negative patients, 8 achieved CR on first induction, 2 later relapsed, and 7 died: 3 were disease-related and 4 (in CR) were due to other causes. Four of 5 CD20-positive patients received at least 80% of the planned L-asparaginase dose, as well as 6 of the 8 CD20-negative patients who achieved CR (P=1.00). Three of the 9 CD20 negative patients had the t(4;11) karyotype. Contrary to the results of Maury *et al.*, we found no correlation between CD20 and CIR (P=0.35) or EFS (P=0.16) among this subgroup; surprisingly, CD20-positive patients in this group appeared to have a longer OS (median not reached *vs*. 9.6 months, P=0.023).

The article by Maury *et al.* was the second to report on the prognostic significance of CD20 in adult BCP-ALL.

Table 1. Multivariate analysis for overall survival, cumulative incidence of relapse, and event-free survival in 119 patients with adult BCP-ALL.

Parameter	Overall Survival		Cumulative Incidence of Relapse		Event-Free Survival	
	P	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)
Age over 40, n=59	0.09	1.76 (0.91-3.41)	0.58	1.27 (0.55-2.94)	0.22	1.46 (0.80-2.67)
Allogeneic stem cell transplant, n=32	0.51	1.29 (0.61-2.74)	0.46	0.67 (0.23-1.93)	0.67	0.85 (0.41-1.78)
CD20 positivity, n=49	0.11	0.57 (0.28-1.13)	0.18	0.55 (0.23-1.32)	0.07	0.56 (0.30-1.05)
Leukocyte count over 30×10^9/L, n=28	0.003	2.89 (1.44-5.81)	0.03	2.71 (1.09-6.75)	0.002	2.76 (1.46-5.21)
Philadelphia chromosome, n=39	0.42	1.36 (0.65-2.86)	0.14	2.11 (0.78-5.70)	0.09	1.83 (0.91-3.69)

The first was by Thomas *et al.* who described their experience with 110 patients treated with the VAD/CVAD regimen and 143 patients treated with the hyper-CVAD protocol.[5] Philadelphia chromosome positive patients were included. They found that CD20 positivity was an independent predictor of earlier relapse and shorter OS. The discrepancy between our results and those of Thomas *et al.* may be primarily due to differences in treatments, especially our intensive use of L-aspiraginase. In recent years, such non-myelotoxic drugs, as adopted from pediatric regimens, have been shown to lead to marked improvements in adult BCP-ALL treatment.[2,4,6] Conflicting results surrounding CD20 expression have also been reported in pediatric BCP-ALL. Borowitz *et al.*[7] found that increased CD20 intensity predicted a poorer EFS among a group of patients treated with POG protocols, while Jeha *et al.*[8] reported that CD20 expression was not an adverse factor with St Jude protocols. Differences in chemotherapy regimens may account for much of the observed differences of CD20 significance.

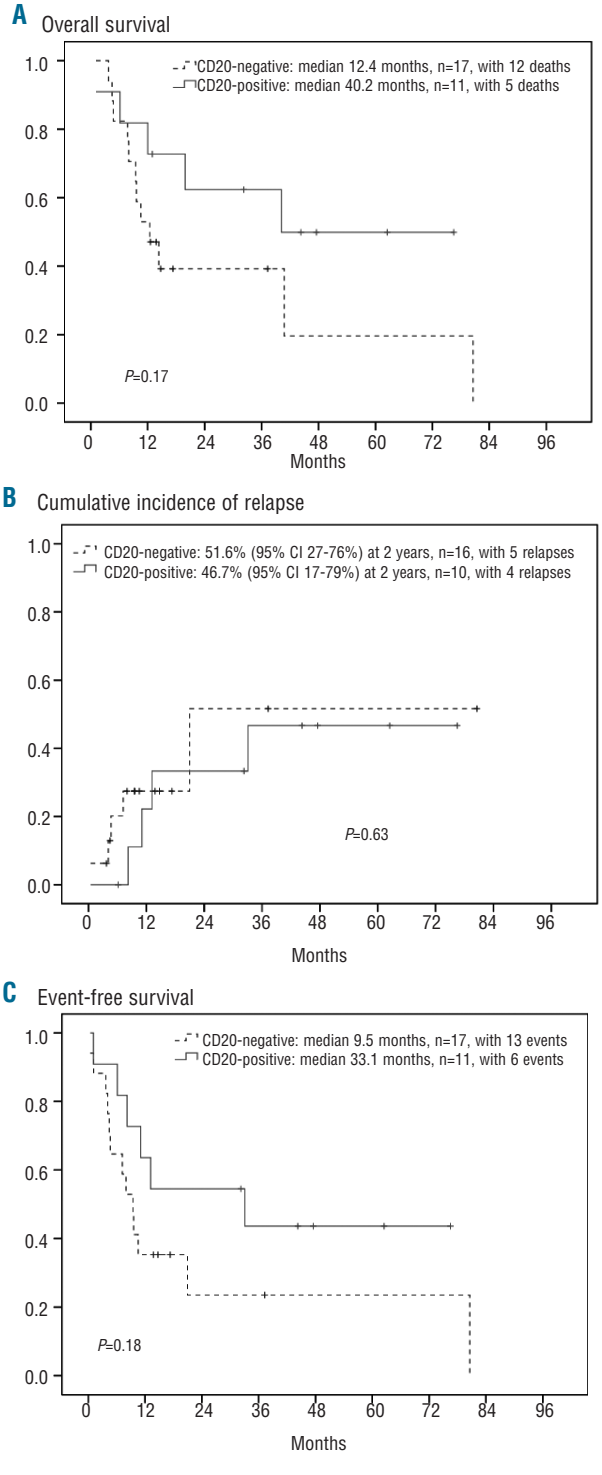

Figure 1. Influence of CD20 expression on the following outcomes in high leukocyte count (>30x10^9/L) adult BCP-ALL patients (includes the Philadelphia chromosome subset): (A) Overall survival, (B) Cumulative incidence of relapse, and (C) Event-free survival.

The study by Maury *et al.* and our study looked at similarly sized cohorts treated with similar protocols. Univariate analysis of both cohorts showed no statistical significance of CD20. However, differences arise in the multivariate and subgroup analyses, even when Philadelphia chromosome positive cases were excluded: Maury *et al.* found poorer outcome associated with CD20 expression while our results showed a trend towards a better outcome among CD20-positive patients. Much of this difference may be attributed to the limited size of both cohorts. Given the variance in results among these studies, the prognostic significance of CD20 expression in B-cell precursor acute lymphoblastic leukemia needs to be reassessed within each treatment regimen, using prospective studies with a larger sample size.

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L-asparaginase for adult CD20 positive B-cell precursor acute lymphoblastic leukemia

The letter by Chang *et al.* raises the interesting question of discrepant results regarding the prognostic impact of CD20 expression in adults with B-cell precursor acute lymphoblastic leukemia. Recently, we evidenced an adverse prognostic impact of CD20 expression on the cumulative incidence of relapse in a multivariate analysis including: (i) CD20 positivity; (ii) age over 45; and (iii) leukocyte count over 30 G/L.[1] Using the same cumulative incidence of relapse endpoint and co-variables but a different statistical model (Kaplan-Meier instead of cumulative incidence curves taking into account deaths in first complete remission as a competing risk), Chang *et al.* found no significant influence of CD20 expression. This conclusion was made in a series of 119 patients apparently treated similarly to a smaller cohort of 85 BCR-ABL negative B-cell precursor acute lymphoblastic leukemia patients reported by the same Canadian group.[2] However, one must note that the clinical results reported in this last study[2] were far better than those reported by Chang *et al.* in their letter. Of note, we incorporated the cut-off of 45 years of age in our analysis since it had been previously identified as the best one to illustrate the negative impact of advanced age in our cohort.[3] Since a cut-off of 35 years of age rather than 45 was previously identified as a significant adverse predictor for overall survival in the published experience of this Canadian group,[2] it might be preferentially included in their multivariate analyses.

Despite these analytical differences, Chang *et al.* show, in contrast to our results, no evidence of any adverse effect associated with CD20 expression in a similarly sized cohort, apparently treated with a quite similar pediatric-inspired protocol. We compared precisely these chemotherapy regimens in order to put forward hypotheses explaining this discrepancy and evidenced two main differences. First, our regimen included cyclophosphamide at different steps of treatment (induction, consolidations, late intensification) while this drug was not used in the schedule mentioned by Chang *et al.* However, one would not easily argue that the use of cyclophosphamide would emphasize the negative impact of CD20 expression in our cohort. In contrast, we observed in the schedule mentioned by Chang *et al.* that around 3-fold higher cumulative doses of L-asparaginase were used in comparison with our protocol (400,000 UI/m^2 *vs.* 144,000 UI/m^2). We hypothesize that this increased use of L-asparaginase may have annihilated the negative impact of CD20 expression. Interestingly, Thomas *et al.* previously reported the same adverse prognostic impact of CD20 expression in adult B-cell precursor acute lymphoblastic leukemia in a series of 110 patients treated with the VAD/CVAD regimen and 143 patients treated with the hyper-CVAD protocol.[4] In their study, CD20 positivity was associated with a higher incidence of relapse (65% *vs.* 42%, P<0.001), lower 3-year complete remission duration rate (20% *vs.* 50%, P<0.001) and lower 3-year overall survival rate (27% *vs.* 40%, P=0.03). Thomas *et al.* further evaluated the significance of CD20 expression with their two sequential regimens of increasing intensity (VAD/CVAD then hyper-CVAD). Whereas intensifying the chemotherapy (from VAD/CVAD to hyper-CVAD) significantly improved the outcome for the CD20 negative group, the outcome for the CD20 positive group was similar regardless of chemotherapy regimen. They suggested that "further intensifying the chemotherapy for CD20 positive precursor B-cell acute lymphoblastic leukemia in a manner other than incorporating monoclonal antibodies (e.g. rituximab) or other targeted agents would be unlikely to improve outcome", but also that "the one potential caveat is the incorporation of asparaginase since it was not a substantial component of either regimen". Indeed, L-asparaginase cumulative doses were 80,000 UI/m^2 in the VAD/CVAD regimen and patients received 2 doses of pegylated asparaginase (2,500 UI/m^2) at the maintenance phase in the hyper-CVAD regimen. In regard to these results obtained from 3 different protocols with their specific chemotherapy components, we fully agree with Chang *et al.* that differences in chemotherapy regimens might account for much of the observed differences of CD20 significance. Further investigations are certainly warranted to determine whether L-asparaginase would be specially needed for adult CD20 positive BCP acute lymphoblastic leukemia.

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