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Paediatrics

Relevance of CD20 antigen expression among paediatric patients with B-lineage acute lymphoblastic leukaemia

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Summary

Literature regarding prognostic relevance of CD20 antigen expression among paediatric B-lineage acute lymphoblastic leukaemia (B-ALL) patients is sparse and contradictory. We analysed clinical laboratory parameters and survival characteristics pertinent to CD20 expression among 224 treatment-naïve paediatric B-ALL patients. 50% patients had CD20 expression (CD20+ B-ALL). There was no difference in the clinical & laboratory presentation and end of induction measurable residual disease (EOI-MRD) status according to CD20 expression. As compared to CD20− B-ALL patients, CD20+ B-ALL patients had two times more relapse (16% vs. 29%, $p = 0.034$), inferior relapse-free survival (79% vs. 66%, $p = 0.025$) but no difference in overall survival (75% vs. 69%, $p = 0.126$). Similar to high-risk NCI status and EOI-MRD positivity, CD20 expression was an independent predictor for inferior relapse-free survival (HR: 1.860, 95% CI: 1.008–3.432, $p = 0.047$). Compared to baseline, there was a significant increase in CD20-expressing EOI-residual blasts among CD20− B-ALL patients (5% vs. 13%, $p = 0.001$). EOI residual blasts of both CD20+ and CD20− patients had three times increased normalized CD20 expression intensity (nCD20), with the intensity among CD20− B-ALL patients reaching the pretreatment nCD20 of CD20+ B-ALL patients (4.9 vs. 3.6, $p = 0.666$). Rituximab can be considered in managing EOI-MRD-positive CD20− B-ALL patients as the residual blasts of these patients have quantitative and qualitative increases in CD20 expression.

KEYWORDS

acute lymphoblastic leukaemia, B lineage, CD20, measurable residual disease, paediatric, rituximab

INTRODUCTION

Precursor B-lineage acute lymphoblastic leukaemia (B-ALL) is the most common paediatric haematological malignancy. With the advent of better disease response assessment tools and chemotherapy regimens, outcomes of paediatric B-ALL patients have been progressively optimistic (nearly 90% survival).[1] Treatment decisions during the management of paediatric B-ALL patients are based on National Cancer Institute (NCI) risk-stratification, recurrent genetic abnormalities, Day 8 peripheral blood (PB) blast clearance and measurable residual disease (MRD) status. However, these risk assessment tools are not foolproof and there are children with B-ALL whose outcomes are still unpredictable. This signifies the need for additional biomarkers that aid in both the outcome prediction and management of children diagnosed with B-ALL.

CD20 is a cell surface antigen that is exclusively expressed among the cells of the B-lymphoid lineage. Literature regarding CD20 expressing B-ALLs in the paediatric age group is sparse and has documented contradictory survival outcomes.[2–7] In the current study, clinical laboratory characteristics and survival outcomes according to baseline CD20 expression status were analysed among the paediatric B-ALL patients managed at our Institute.

MATERIALS AND METHODS

This study was conducted at a regional cancer centre. With Institutional Ethics Committee approval, consecutive treatment-naïve paediatric patients (age up to 18 years) diagnosed with B-ALL between 1 January 2018 and 31 December 2021 were recruited. Diagnosis of B-ALL was by the morphological evaluation of Leishman-stained PB and bone marrow aspiration (BMA) smears, followed by flow cytometric immunophenotyping (FCI). The clinical and laboratory parameters of these patients were compiled from the hospital's electronic records. All patients were treated and risk stratified at the end of induction as per Indian Collaborative Childhood Leukemia (ICiCLe) protocol and were followed up until 30 June 2023.[8]

Flow cytometric immunophenotyping at diagnosis

Our eight-tube, ten-colour cocktail for diagnostic FCI and DNA index (DI) analysis is depicted in Table S1. BMA samples for FCI and DI analysis were processed as described previously.[9,10] A minimum of 100 000 events were acquired per tube in the Beckman Coulter (BC) Navios EX flow cytometer. The list mode data files generated were analysed in BC Kaluza software (version 2.0) using in-house developed sequential gating-based analysis templates. The expression profile for each antigen in our panel was analysed based on AIEOP-BFM recommendations of 2016.[11] The intensity of antigen expression was defined by the geometric mean (GM) of immunofluorescence of that antigen.

In Tube 2 of our FCI panel (refer Table S1), CD19-positive and CD45-neg/dim gated events showing aberrant overexpression/underexpression/asynchronous expression in the combination dot plots for CD19, CD45, CD10, CD34, CD38, CD20, CD73, CD86, CD123 and CD58 were considered as leukaemic B lymphoblasts (henceforth referred as blasts). The presence of intracytoplasmic CD79a and the absence of cytoplasmic CD3 and myeloperoxidase in these blasts were confirmed in Tube 6.

For analysing CD20 expression among the blasts, normal B lymphocytes (low forward and side scatter, CD45 moderate, CD19 moderate, CD20 moderate and CD34 negative) and non-B-lymphoid cells (low forward and side scatter, CD45 moderate, CD19 negative, CD20 negative and CD34-negative events) in the sample served as internal positive and negative controls respectively. The proportion of blasts that had CD20 expression (CD20%) above the non-B lymphocytes was considered positive for CD20 expression. Samples with >20% leukaemic blasts with CD20 expression were considered CD20 expressers (CD20+ B-ALL) and samples with ≤20% leukaemic blasts with CD20 expression were considered CD20 non-expressers (CD20− B-ALL).[2–5,12–15] The intensity of CD20 antigen expression in the blasts was calculated as normalized CD20 expression (nCD20), which was the ratio between CD20-GM of all blasts to CD20-GM of non-B-lymphoid cells.

Baseline evaluation for recurrent genetic abnormalities

At diagnosis, the presence of *BCR::ABL1*, *ETV6::RUNX1*, intrachromosomal amplification of chromosome 21 (iAMP21), *TCF3::PBX1* and *KMT2A* rearrangement were analysed by interphase fluorescence in situ hybridization (iFISH) as per our published methodology.[16] Ploidy was assessed by conventional karyotyping and by DNA index (DI).[17] The presence of *BCR::ABL1*, *KMT2A* rearrangement, iAMP21, near-haploidy (24–29 chromosomes or DI 0.55–0.69), low-hypodiploidy (31–39 chromosomes or DI 0.70–0.88) and near-triploidy (66–80 chromosomes or DI 1.40–1.79) were considered high-risk cytogenetics.[17,18] During the induction phase of treatment, presence of ≥1000 blasts/μL in the PB on Day 8 of treatment (prednisolone monotherapy) was considered Day 8 blast not cleared status (D8BNC).

End of induction-MRD assessment

At the end of induction (EOI), the first pull bone marrow aspiration sample was processed using bulk-lyse stain-wash protocol and stained with a single-tube, 10-colour antibody cocktail (same as Tube 2 used in the diagnostic FCI). MRD was evaluated and calculated using in-house developed analysis templates as described in our previous publication.[9] The presence of >5% blasts by morphology in the bone marrow aspiration was considered induction failure.

Statistics

Statistical analysis was performed using Microsoft Excel 2016, the Statistical Package for Social Sciences (SPSS version 23; IBM, Armonk, NY), and MedCalc version 14.8.1. For intergroup comparison of parameters between CD20+ and CD20− B-ALL patients, Chi-squared and Mann–Whitney *U* tests were used for categorical (sex, ICiCLe risk groups, cytogenetics, baseline CNS involvement, MRD status, relapse/remission status) and continuous variables (haemoglobin, WBC count, platelet count, MRD%, CD20%, and nCD20) respectively. The median duration of follow-up was calculated by reverse Kaplan–Meier method. The magnitude of difference in the normalized CD20 expression and the percentage of blasts expressing CD20 antigen at diagnosis and at EOI was calculated as percentage of delta change. Overall survival (OS) and relapse-free survival (RFS) were calculated from the date of diagnosis to the date of last follow-up/death and disease relapse (after achieving remission) respectively. Wilcoxon's signed-rank test was used to assess differences in the CD20% and nCD20

between the baseline and EOI residual blasts. Differences in OS and RFS between CD20+ and CD20− B-ALL patient groups were evaluated by the Kaplan–Meier survival analysis with log-rank test. The risk incurred by CD20 expression, NCI high-risk status, ICiCLe risk groups, high-risk cytogenetics, D8BNC status and EOI-MRD positivity towards OS and RFS were assessed by univariate and multivariate analysis using Cox proportional hazards model. The receiver operator curve (ROC) was used to identify the cut-offs of CD20% and nCD20 that were discriminatory for inferior survival and relapse. All tests were two-tailed and a p-value of ≤0.05 was considered significant at a 95% confidence interval.

RESULTS

Among 224 treatment-naïve B-ALL patients diagnosed during the study time frame, 50% ($n = 111$) had CD20 expression in >20% blasts. The clinical and laboratory features of our CD20+ and CD20− B-ALL patients are depicted in Table 1. These 224 patients did not have any significant propensity toward any of the EOI ICiCLe risk groups (ICiCLe standard risk: 79 patients [35%], ICiCLe intermediate risk: 42 patients [19%] and ICiCLe high risk: 103 patients [46%], $p = 0.155$). Out of these 224 patients, 13 (6%) were not willing for treatment, these included 2 ICiCLe high-risk, 4 ICiCLe intermediate-risk and 7 ICiCLe standard-risk patients.

TABLE 1 Comparison of clinical and laboratory parameters of paediatric B-ALL patients based on CD20 expression.

Parameters	All patients ($n = 224$)	CD20 expression		*p* value
		CD20− B-ALL ($n = 113$)	CD20+ B-ALL ($n = 111$)	CD20− B-ALL vs. CD20+ B-ALL
Median (IQR) % of blasts expressing CD20	19 (5–68)	4.9 (2–10)	69 (40–92)	**<0.001**
Median (IQR) nCD20 expression	2.2 (1.3–5.9)	1.3 (1.1–1.8)	5.9 (2.8–18)	**<0.001**
Median (IQR) age in years	5 (3–12)	5 (3–10)	5 (3–12)	0.972
Sex (Male: Female)	1.5:1	1.35:1	1.7:1	0.397
Median (IQR) Hb in g/L	70 (53–85)	67 (52–82)	72 (57–89)	0.161
Median (IQR) WBC count $\times 10^9$/L	9.6 (3.7–31)	10.7 (4.2 to 32)	8.2 (3.3 to 31)	0.203
Median (IQR) platelet count $\times 10^9$/L	45 (22–73)	51 (27–82)	39 (20–67)	0.053
Splenomegaly (%)	114 of 224 (51)	55 of 113 (49)	59 of 111 (53)	0.514
Hepatomegaly (%)	97 of 224 (43)	43 of 113 (38)	54 of 111 (49)	0.111
Lymphadenopathy (%)	111 of 224 (49)	52 of 113 (46)	59 of 111 (53)	0.321
CNS involvement at diagnosis (%)	4 of 211 (2)	1 of 106 (1)	3 of 105 (3)	0.308
BCR::ABL1 positive (%)	11 of 212 (5)	4 of 109 (4)	7 of 103 (7)	0.305
ETV6::RUNX1 positive (%)	22 of 212 (10)	14 of 109 (13)	8 of 103 (8)	0.226
KMT2A rearranged (%)	5 of 212 (2)	4 of 109 (4)	1 of 103 (1)	0.196
TCF3::PBX1 positive (%)	14 of 212 (7)	8 of 109 (7)	6 of 103 (6)	0.657
Diploidy (%)	150 of 212 (71)	76 of 109 (70)	74 of 103 (72)	0.764
High-hyperdiploidy (%)	44 of 212 (21)	26 of 109 (24)	18 of 103 (18)	0.310
Low-hypodiploidy/near-triploidy (%)	2 of 212 (1)	1 of 109 (1)	1 of 103 (1)	1.000
Near-tetraploidy (%)	4 of 212 (2)	1 of 109 (1)	3 of 103 (3)	0.358
High-risk genetic abnormalities[a] (%)	18 of 212 (8)	9 of 109 (8)	9 of 103 (9)	1.000
Day 8 circulating blast not cleared (%)	20 of 211 (10)	14 of 106 (13)	6 of 105 (6)	0.063
Induction death (%)	6 of 211 (3)	2 of 106 (2)	4 of 105 (4)	0.401
Induction failure (%)	2 of 205 (1)	1 of 104 (1)	1 of 101 (1)	0.983
EOI-MRD positivity (%)	77 of 203 (38)	37 of 104% (36)	40 of 99 (40)	0.479
ICiCLe standard risk (%)	79 of 224 (35)	46 of 113 (41)	33 of 111 (30)	0.086
ICiCLe intermediate risk (%)	42 of 224 (19)	17 of 113 (15)	25 of 111 (22)	0.152
ICiCLe high risk (%)	103 of 224 (46)	50 of 113 (44)	53 of 111 (48)	0.599
Relapse	45 of 205 (22)	16 of 104 (16)	29 of 101 (29)	**0.034**

Abbreviations: EOI-MRD, end of induction-measurable residual disease; ICiCLe, Indian Collaborative Childhood Leukaemia.

[a]High-risk genetic abnormalities include: *BCR::ABL1* fusion, *KMT2A* rearrangement, iAMP21 and low-hypodiploidy/near-triploidy. None of the patients were positive for iAMP21. Cytogenetic evaluation was not done in 12 patients who did not undergo treatment. All patients were treated and risk stratified at the end of induction as per the ICiCLe protocol.

Among the remaining 211 patients who were treated, six (3%) patients died during induction and two (1%) patients incurred induction failure.

Among 203 patients where EOI-MRD was assessed, a median of 3 million (95% CI: 2.9 to 3.5 million) events were acquired. EOI-MRD was positive in 38% of the cohort (77 of 203 patients) and there was no significant difference in the frequency of EOI-MRD positivity between CD20+ and CD20− B-ALL patients (40% vs. 36%, $p = 0.479$).

The EOI residual blasts among these 77 patients had significantly upregulated median nCD20 intensity as compared to the paired baseline nCD20 expression (5.1 vs. 2.4, $p < 0.001$). This difference in the median nCD20 intensity between the EOI residual blasts and baseline blasts was significant in both CD20− B-ALL (3.6 vs. 1.3, $p < 0.001$) and CD20+ B-ALL patients (14.1 vs. 4.9, $p = 0.004$) patients. Besides, there was no difference in the median nCD20 between the pretreatment blasts of CD20+ patients and the residual blasts of CD20− patients (4.9 vs. 3.6, $p = 0.666$). Compared to baseline, there was a significant increase in the median percentage of residual blasts expressing CD20 antigen only among CD20− B-ALL patients (5% vs. 13%, $p = 0.001$), but not among CD20+ B-ALL patients (64% vs. 80%, $p = 0.320$). Table 2 compares the differences and the magnitude of change in the normalized CD20 expression and the percentage of blasts expressing CD20 antigen at diagnosis and at the EOI among EOI-MRD-positive patients.

The median follow-up of our patients was 42 months (95% CI: 39–45 months), with 41 months (95% CI: 39–46 months) median follow-up among CD20− B-ALL patients and 44 months (95% CI: 38–48 months) median follow-up among CD20+ B-ALL patients. During follow-up, 22% (45 of 205) patients had a relapse (isolated medullary relapse in 24 patients, isolated CNS relapse in 10 patients, concurrent medullary & CNS relapse in nine patients, and concurrent medullary and non-CNS extra-medullary relapse in two patients). The overall frequency of relapse was significantly higher among CD20− B-ALL patients than CD20+ B-ALL patients (16% vs. 29%, $p = 0.034$). The 12-, 24-, 36- and 48-month-specific cumulative incidence of relapse among CD20− B-ALL and CD20+ B-ALL patients were 4% vs. 8%, 9% vs. 20%, 17% vs. 30% and 21% vs. 34% respectively (refer Figure 1).

The 4-year RFS and OS of the entire cohort were 73% and 72% respectively. CD20+ B-ALL patients had inferior 4-year RFS than CD20− B-ALL patients (66% vs. 79%, $p = 0.025$). However, there was no significant difference in the 4-year OS between both the groups (69% vs. 75%, $p = 0.126$), refer to Figure 2.

On ROC analysis, baseline nCD20 intensity of >1.85 was associated with significantly higher relapse with 78% sensitivity, 48% specificity and area under the curve of 0.614 ($p = 0.013$). Also, baseline CD20 expression seen in >7.3% blasts were associated with significantly higher relapse (area under the curve = 0.599, sensitivity = 89%, specificity = 39% and $p = 0.02$), refer to Figure 3. However, there were no ROC-defined cut-offs for nCD20 and the percentage of CD20 expressing blasts that could significantly segregate patients

TABLE 2 Comparison of differences in the normalized CD20 expression and the percentage of blasts expressing CD20 antigen at diagnosis and end of induction.

Patient group	Median (IQR) nCD20 expression among blasts				Median (IQR) % of blasts expressing CD20 antigen			
	At diagnosis	At EOI	Delta change from diagnosis	*p*-value	At diagnosis	At EOI	Delta change from diagnosis	*p*-value
All patients ($n = 77$)	2.4 [1.3–5.4]	5.1 [2.5–24.2]	112%	**<0.001**	22 [5–67]	35.48 [4–90]	61%	0.118
CD20+ B-ALL patients ($n = 40$)	4.9 [2.8–11.2]	14.1 [3.2–30.3]	188%	**0.004**	64 [34–91]	80 [8–97]	25%	0.320
CD20− B-ALL patients ($n = 37$)	1.3 [1.0–1.7]	3.6 [1.6–13.4]	176%	**<0.001**	5 [2–8]	13 [0.65–68]	160%	**0.001**

Abbreviations: EOI, end of induction; nCD20, normalized CD20.

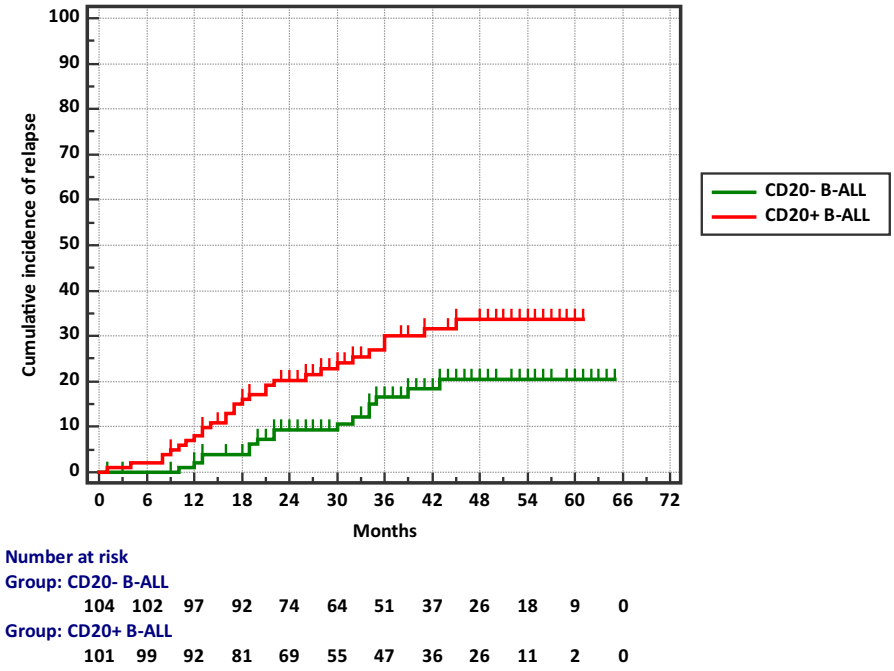

FIGURE 1 Kaplan–Meier plots depicting cumulative incidence of relapse among paediatric B-ALL patients according to CD20 expression status at diagnosis.

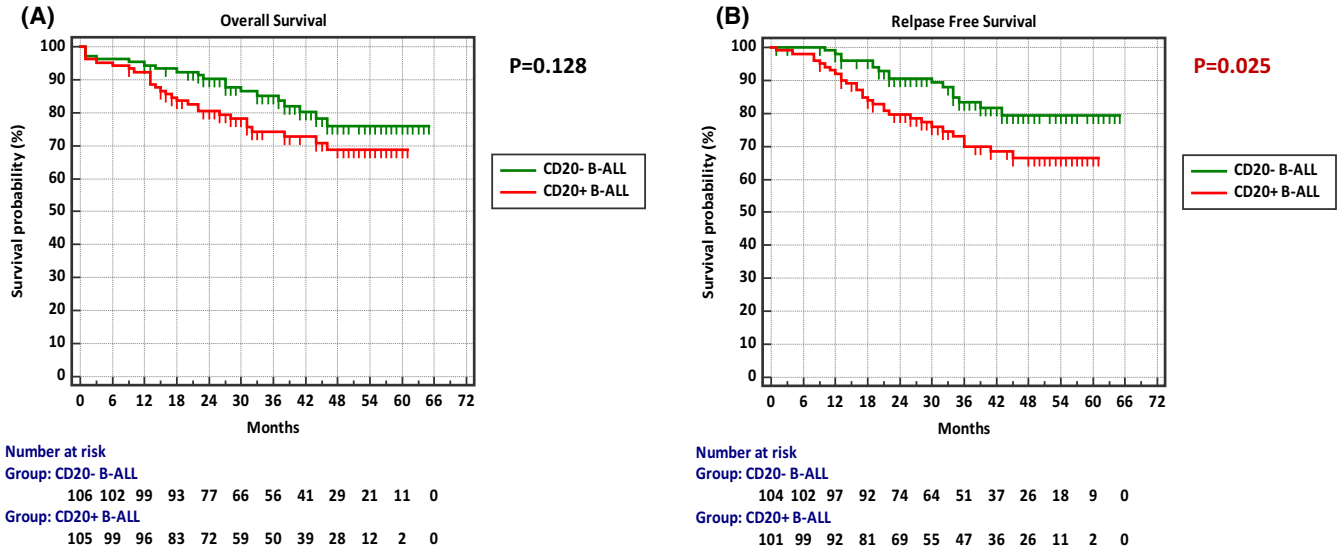

FIGURE 2 Kaplan–Meier plots comparing overall survival (A) and relapse-free survival (B) among paediatric B-ALL patients according to CD20 expression status at diagnosis.

with inferior survival ($p > 0.05$). Univariate and multivariate analyses regarding the impact of NCI high-risk, EOI ICiCLe risk groups, CD20 expression, high-risk cytogenetics, D8BNC and EOI-MRD positivity on the OS and RFS are depicted in Table 3.

DISCUSSION

Nearly half of our paediatric patients with B-ALL had CD20 expression, which is similar to the frequency reported in most of the literature, but is lower than the frequency reported by Borowitz et al. (67%) and Solano-Genesta et al. (62%).[2–6,12,19] According to available literature and our results, the baseline clinicolaboratory features, including the frequency of recurrent genetic abnormalities and EOI-MRD status, do not differ between paediatric B-ALL patients concerning their CD20 expression status.[2,4]

There is limited literature regarding the prognostic relevance of CD20 expression among paediatric B-ALL patients, and the survival outcomes documented are contradictory.[2–6] According to Borowitz et al. and Aref et al., CD20 expression

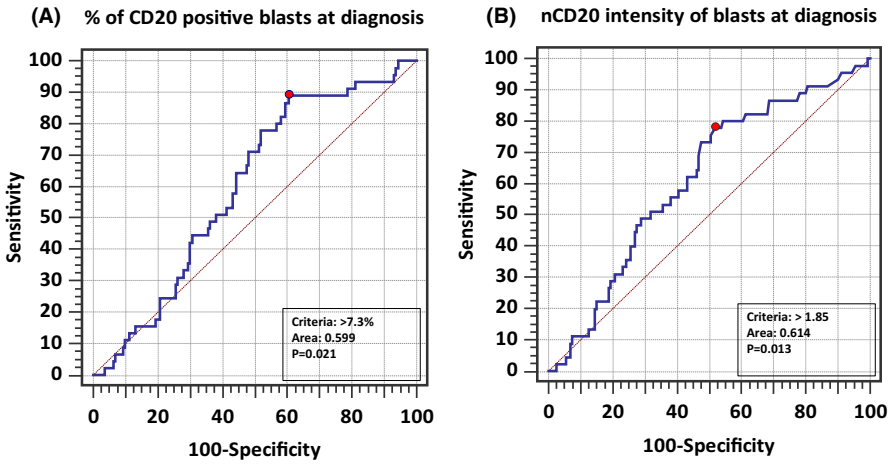

FIGURE 3 Receiver operator curves to determine cut-offs for a percentage of blasts expressing CD20 at diagnosis (A) and normalized CD20 (nCD20) expression intensity (B) using disease relapse as a classification variable. The red dots represent the cut-off points identified by the Youden index.

TABLE 3 Univariate and multivariate Cox regression analyses for overall and relapse-free survival.

	OS			RFS		
Variables	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Univariate analysis						
CD20-positive status	1.562	0.872–2.798	0.133	1.971	1.070–3.630	**0.029**
NCI high risk	2.069	1.160–3.689	**0.014**	2.186	1.209–3.951	**0.010**
ICiCLe high risk	1.657	0.925–2.967	0.090	1.695	0.933–3.077	0.083
ICiCLe intermediate risk	1.692	0.878–3.263	0.116	1.439	0.712–2.910	0.311
ICiCLe standard risk	0.310	0.139–0.692	**0.004**	0.376	0.175–0.808	**0.012**
High-risk cytogenetics	0.257	0.035–1.867	0.179	0.274	0.038–1.997	0.274
Day 8 blast not cleared	0.805	0.289–2.245	0.679	1.121	0.442–2.842	0.810
EOI-MRD positive	2.780	1.484–5.208	**0.001**	2.246	1.247–4.045	**0.007**
Multivariate analysis						
NCI high risk	2.073	1.111–3.865	**0.022**	2.037	1.124–3.692	**0.019**
EOI-MRD positive	2.636	1.405–4.946	**0.003**	2.118	1.174–3.818	**0.013**
CD20-positive status	-	-	-	1.860	1.008–3.432	**0.047**

Abbreviations: EFS, event-free survival; EOI-MRD, end of induction-measurable residual disease; ICiCLe, Indian Collaborative Childhood Leukaemia; NCI, National Cancer Institute; OS, overall survival; RFS, relapse-free survival.

among paediatric B-ALL patients confer inferior OS and short EFS.[6] However, Jeha et al. have documented better survival.[2] The data from Rahul et al. and Solano-Genesta et al. do not demonstrate any prognostic relevance rendered by CD20 expression among these patients.[3,4] These differences in survival outcomes might be due to heterogeneity in the patient cohort size, age group of patients included, frequency of underlying recurrent genetic abnormalities and treatment protocols used. According to our results, CD20+ B-ALL patients had two times higher frequency of disease relapse than CD20− B-ALL patients. In addition to NCI high-risk status and EOI-MRD positivity, which are well-established predictors of inferior outcomes among paediatric B-ALL patients, CD20 expression in >20% blasts was also an independent predictor of inferior RFS among our patients. However, baseline CD20 expression in >20% of blasts did not influence the OS of our patients.

In the majority of literature that has analysed the prognostic relevance of CD20 expression in B-ALL, the patients were identified based on the presence of CD20 antigen in >20% blasts.[2–5,20,21] The prognostic relevance of B-ALL patients with <20% blasts expressing CD20 antigen is largely unexplored. Recently, Tian et al. and Marks et al. have documented that CD20 expression in nearly 11% of blasts is also associated with inferior outcomes.[22,23] In our cohort, the presence of CD20 antigen in >7.3% blasts was associated with significantly inferior RFS with 89% sensitivity, but with only 39% specificity.

Both Serbanica et al. and Tian et al. have documented adverse prognoses in B-ALL patients based on the intensity of CD20 antigen expression rather than the percentage of blasts

expressing CD20.[7,23] Tian et al. have documented that a <19.98 mean fluorescence intensity (MFI) for CD20 in blasts (determined as the ratio of blast's CD20 MFI to that of isotype control's MFI) as an independent predictor for inferior OS and progression-free survival.[23] However, their cohort of 206 B-ALL patients comprised only 100 patients under the age of 14 years, and paediatric age-specific survival data were not available. According to Serbanica et al., Pacific Blue fluorochrome-specific CD20 MFI of >8.08 was associated with inferior RFS and OS among their cohort of 114 paediatric B-ALL patients (0–17 years of age).[7] The modalities for determining the intensity of CD20 expression in these two studies might not be widely applicable due to heterogeneity in instrumentation, sample processing techniques, choice of fluorochromes and the need for isotype controls.

Our method of determining the intensity of CD20 expression as nCD20 is more practical, as it is not instrument or fluorochrome specific, does not incur any additional cost and is immediately applicable across all laboratories. According to our data, nCD20 of >1.85 in blasts was associated with inferior RFS with 78% sensitivity. However, the specificity of this cut-off was only 48%, limiting its practical utility.

Rituximab is a monoclonal antibody targeting the CD20 protein expressed on the surface of B-lineage cells.[21] Incorporating rituximab in the treatment of CD20+ B-ALL patients has yielded optimistic results.[15,19–21,24] In these protocols, rituximab was given only to those patients who were expressing CD20 antigen in more than 20% of blasts at diagnosis.[15,21] Quantitative and qualitative upregulation of CD20 expression in the blasts after steroid exposure is a well-known phenomenon.[12,25] In this context, there was nearly three times significant upregulation of nCD20 intensity of EOI residual blasts in both CD20+ and CD20− patients of ours, which is in line with Dowrzak et al.'s observation.[12]

Though the increase in the nCD20 intensity of residual blasts of our patients was irrespective of the baseline CD20 expression status, the increase in the percentage of residual blasts expressing CD20 was significant only among the up-front CD20− B-ALL patients (5% vs. 13%, $p = 0.001$). Though the percentage of EOI residual blasts expressing CD20 among these up-front CD20− patients did not cross the conventional 20% threshold, there was significant upregulation of their nCD20 intensity, reaching up to the pretreatment nCD20 of CD20+ patients (4.9 vs. 3.6, $p = 0.666$). Steroid-induced upregulation of CD20 expression in the blasts and the ensuing enhanced cytotoxic potential of rituximab is a known phenomenon.[12] In this context, our results open avenues for considering rituximab in the management of up-front CD20− B-ALL patients who are EOI-MRD positive, as the residual blasts of these patients also have significantly upregulated the intensity of CD20 expression.

CONCLUSION

CD20 antigen is expressed in 50% of paediatric patients with B-ALL and is an independent predictor of inferior RFS. Among baseline CD20− B-ALL patients, the EOI residual blasts have both quantitative and qualitative increases in CD20 expression. Hence, rituximab can be considered in the management of baseline CD20− B-ALL patients who are EOI-MRD positive.

AUTHOR CONTRIBUTIONS

Karthik Bommannan conceptualized the idea, analysed all the data and wrote the manuscript; Jhansi Rani analysed cases; Venkatraman Radhakrishnan and Shirley Sundersingh assisted in writing and editing the manuscript.

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Nil.

CONFLICT OF INTEREST STATEMENT

No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article. None of the authors have any significant financial interest, consultancy or other relationship with products, manufacturer(s) of products or providers of services related to this manuscript. All authors have agreed to the current version of the manuscript being submitted.

DATA AVAILABILITY STATEMENT

Data pertinent to the current manuscript will be available upon reasonable request and after clearance from our Institute's ethics committee.

ETHICS STATEMENT

Obtained (IEC/2021/July 01).

PATIENT CONSENT STATEMENT

NA.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

NA.

CLINICAL TRIAL REGISTRATION

NA.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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