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Original Article

Expression of CD25 is a specific and relatively sensitive marker for the Philadelphia chromosome *(BCR-ABL1)* translocation in pediatric B acute lymphoblastic leukemia

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Abstract: Background: Precursor B acute lymphoblastic leukemia (B-ALL) is the most common cancer in children and overall, has an excellent prognosis. However, the Philadelphia chromosome translocation (Ph+), t(9;22)(q34;q11), is present in a small subset of patients and confers poor outcomes. CD25 (IL-2 receptor alpha chain) expression has been associated with Ph+ B-ALL in adults, but no similar study has been performed in pediatric B-ALL. Methods: A retrospective analysis of 221 consecutive pediatric patients with a diagnosis of B-ALL (blood and/or bone marrow) from 2009 to 2012 was performed to determine an association between Ph+ B-ALL and CD25 expression. A threshold of 25% was used to define positive cases for CD25 expression by flow cytometry. Results: There were 221 patients with a diagnosis of B-ALL ranging from 2 to 22 years (median, 6 years). Eight (3.6%) B-ALL patients were positive for the Philadelphia chromosome translocation (Ph+ B-ALL) and 213 were negative (Ph-negative B-ALL). CD25 expression was observed in 6 of 8 (75%) Ph+ B-ALL patients and 6 of 213 (2.8%) Ph-negative B-ALL patients. CD25 expression was significantly higher in Ph+ B-ALL compared to Ph-negative B-ALL, with median CD25 expression of 64% (range 0-93%) and 0.1% (range 0-91%), respectively ($P \leq 0.0002$). Therefore, CD25 expression as a predictor of Ph+ B-ALL had 75% sensitivity, 97% specificity, 50% positive predictive value and 99% negative predictive value. Conclusions: CD25 expression is a specific and relatively sensitive marker for the identification of Ph+ B-ALL in the pediatric population.

Keywords: CD25, BCR-ABL, Philadelphia chromosome, Ph+, t(9;22), B acute lymphoblastic leukemia, B-ALL, Ph+ B-ALL, flow cytometry, pediatric

Introduction

Precursor-B-acute lymphoblastic leukemia (B-ALL) is the most common cancer in children [1]. Overall, childhood B-ALL has an excellent prognosis, with complete remission achieved in over 95% and long-term event-free survival of approximately 85% [2-7]. The Philadelphia chromosome (Ph+) or *BCR-ABL1* fusion gene is the result of a t(9;22)(q34;q11) translocation and is present in 20-30% of adults with B-ALL but only in 3-5% of childhood B-ALL [8-10]. Both pediatric and adult Ph+ B-ALL patients are more difficult to treat with higher rates of relapse and worse overall survival [10-12]. Early identification of Ph+ B-ALL is important for early initiation of a tyrosine kinase inhibitor in combination with conventional chemotherapy.

Expression of CD25 (interleukin-2 receptor alpha chain) by flow cytometric analysis has been shown to have an association with Ph+ B-ALL in adult leukemia studies [13-15], suggesting that CD25 could be used as a surrogate marker for adult Ph+ B-ALL. However, no such study has been conducted in pediatric B-ALL.

Materials and methods

The study was approved by the Institutional Review Board of Baylor College of Medicine,

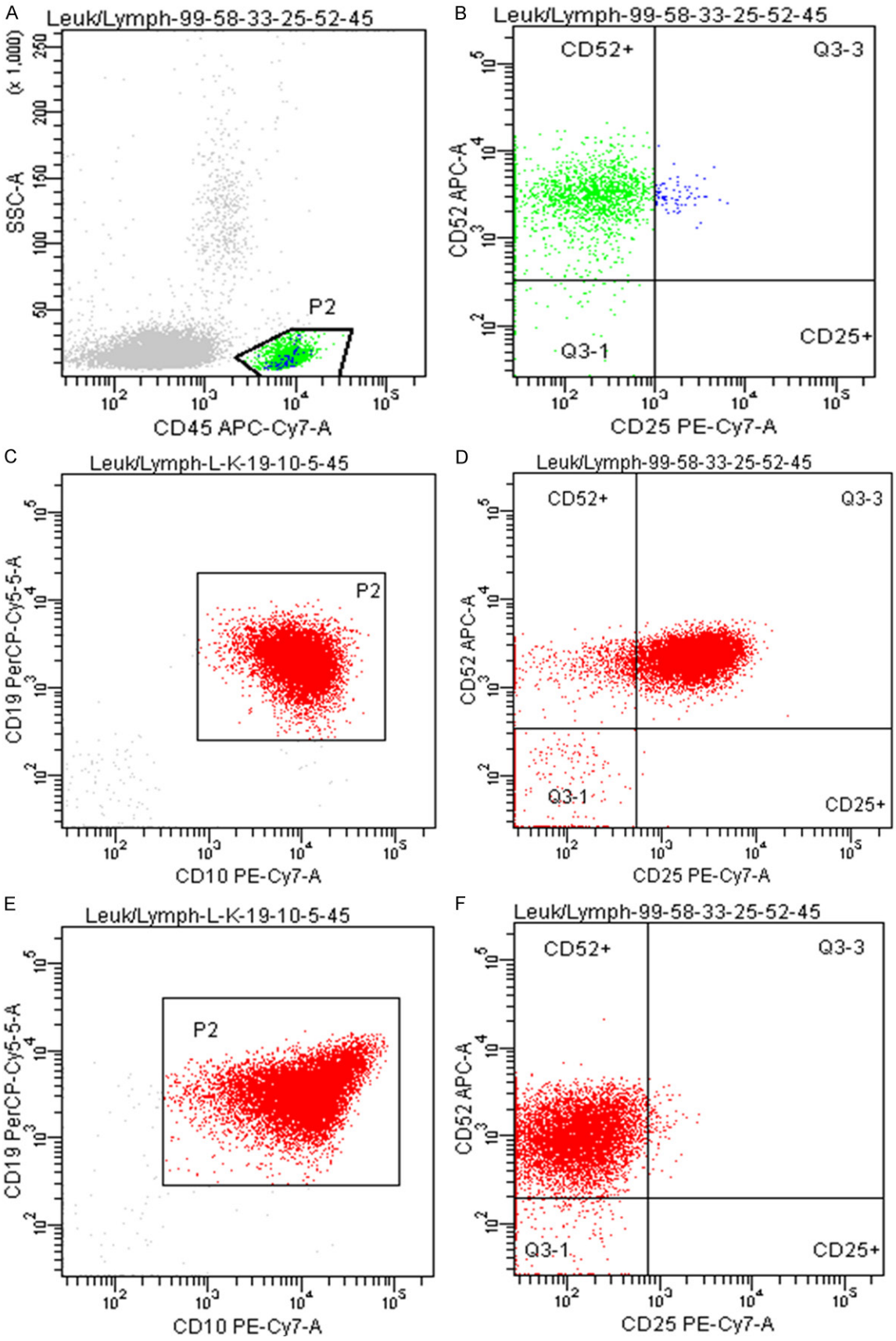
A
Leuk/Lymph-99-58-33-25-52-45
SSC-A
(x 1,000)
P2
CD45 APC-Cy7-A
B
Leuk/Lymph-99-58-33-25-52-45
CD52+
Q3-3
CD52 APC-A
Q3-1
CD25+
CD25 PE-Cy7-A
C
Leuk/Lymph-L-K-19-10-5-45
P2
CD19 PerCP-Cy5-5-A
CD10 PE-Cy7-A
D
Leuk/Lymph-99-58-33-25-52-45
CD52+
Q3-3
CD52 APC-A
Q3-1
CD25+
CD25 PE-Cy7-A
E
Leuk/Lymph-L-K-19-10-5-45
P2
CD19 PerCP-Cy5-5-A
CD10 PE-Cy7-A
F
Leuk/Lymph-99-58-33-25-52-45
CD52+
Q3-3
CD52 APC-A
Q3-1
CD25+
CD25 PE-Cy7-A

Figure 1. Representative plots used in CD25 analysis. A, B: Gating strategy for CD25 analysis. A: Normal lymphocytes (P2) were gated on a CD45 vs side scatter dot plot. B: Normal lymphocytes (P2) from plot A show a sub-population (in blue) that expresses CD25. This quadrant is used to identify the percentage of gated blasts that express CD25. C, D: Representative example of positive CD25 expression in B-ALL (Ph-negative). C: Gated lymphoblasts (P2) shown on a CD10 vs. CD19 plot. D: 90% of the gated lymphoblasts express CD25, which is considered positive. E, F: Representative example of negative CD25 expression in B-ALL (Ph-negative). E: Gated lymphoblasts (P2) on CD10 vs CD19 plot. F: 0.7% of gated lymphoblasts express CD25, which is considered negative.

Table 1. Association between CD25 expression and the Philadelphia chromosome (*BCR-ABL1*) translocation in pediatric B-ALL

BCR-ABL1 Status	CD25-	CD25+	Median CD25 Expression (%)	*P*
Ph-positive	2	6	64 (0-93)	0.00018
Ph-negative	207	6	0.10 (0-91)	

Houston, Texas. A retrospective analysis of consecutive patients at Texas Children's Hospital with a new diagnosis of B-ALL over a three-year period (May 2009 to June 2012) was performed. The diagnosis of B-ALL was made based on criteria published by the World Health Organization 2008 [16]. Flow cytometric, cytogenetic, and FISH analyses were performed in all cases. Corresponding results of RT-PCR analysis of *BCR-ABL1* transcripts were collected from medical records if available. Cases of infantile B-ALL (≤ 1 year) were excluded from analysis. Of note, all seven cases of infantile B-ALL had *MLL* gene rearrangement by FISH analysis.

Multi-parametric flow cytometry

A six-color multi-parametric flow cytometric analysis was performed on fresh cells according to standard protocol. The panel of 33 antibodies was performed using combination of FIT-C/PE/PerCP-Cy5.5/PE-Cy7/APC/APC-H7 as follows: CD7/CD2/CD3/CD8/CD4/CD45, Lambda/Kappa/CD19/CD10/CD5/CD45, CD15/CD42+61/CD34/CD38/CD11b/CD45, CD64/CD16+56/CD13/CD14/CD117/CD45, CD71/Glycophorin A/HLA-DR/CD20/CD22/CD-45, CD99/CD58/CD33/CD25/CD52/CD45

All antibodies were purchased from Becton-Dickinson (San Jose, CA, USA), except anti-CD52-APC (BioLegend, SanDiego, CA). CD25 (Clone M-A251, BD Biosciences, San Jose, CA, USA) was used for this study. The stained samples were run on BD-FACS Canto cytometer and analyzed with BD DIVA version 6.1.3 software (Becton-Dickinson, Franklin Lakes, New Jersey). Approximately 15,000 total events were acquired.

CD25 expression was reported as the percentage of CD25 positive lymphoblasts. CD25/CD52 dot plots were used to determine positive and negative populations by using normal lymphocytes as a reference to set the quadrants (**Figure 1A** and **1B**). In the majority of cases, two distinct populations of residual normal CD25+ and CD25- lymphocytes were identified, and the quadrants were set between these two populations.

Statistical analysis

IBM-SPSS Statistics v.21 was used for all statistical analysis. CD25 expression in Ph+ B-ALL was compared with Ph-negative B-ALL using the Mann-Whitney U test (MW). A receiver-operating characteristic (ROC) curve was generated to determine optimal cutoff values to distinguish Ph+ B-ALL from Ph-negative B-ALL, with a cutoff of 25% for CD25 expression to indicate a CD25-positive case.

Results

There were 221 pediatric B-ALL patients diagnosed in our hospital from May 2009 to June 2012, which was comprised of 118 males (53.4%) and 103 females (46.6%), and ranged in age from 2 to 22 years (median 6 years). Of note, two patients were older than 18 years old, 19 and 22, respectively, and both were negative for CD25 expression and were negative for *BCR-ABL1* translocations. Eight (3.6%) B-ALL patients were *BCR-ABL1* positive (Ph+ B-ALL), and 213 patients were *BCR-ABL1* negative (Ph-negative B-ALL), by both chromosome and FISH analysis.

CD25 expression by flow cytometry was analyzed in newly diagnosed B-ALL patients (**Figure 1C-F**). Positive CD25 expression, using 25% as a cutoff, was observed in 6 of 8 (75%) Ph+ B-ALL patients [CD25+ Ph+ B-ALLs] with a median CD25 expression of 80.5% (range

26-93%). Among 2 Ph+ B-ALL cases with negative CD25 expression [CD25- Ph+ B-ALLs], CD25 expression was 0% and 6%, respectively. Positive CD25 expression was identified in 6 cases of 213 (2.8%) of Ph-negative B-ALL patients [CD25+ Ph-negative B-ALL] with a median CD25 expression of 63% (range 35-91%). Overall, CD25 expression was significantly higher in Ph+ B-ALL compared to Ph-negative B-ALL, with median CD25 expression of 64% (0-93%) and 0.1% (0-91%) respectively ($P \leq 0.0002$) (**Table 1**). CD25 expression in Ph+ B-ALL had 75% sensitivity, 97% specificity, 50% positive predictive value (PPV) and 99% negative predictive value (NPV). Of note, we identified 4 patients who had relapsed B-ALL, with time of relapse ranging from 4 to 30 months, and all cases were Ph-negative B-ALL that showed negative CD25 expression in both original and relapse samples.

Discussion

Previous studies have been conducted to identify an association between CD25 expression and Ph+ B-ALL in adult populations. Our study is the first to specifically focus on pediatric patients [13-15, 17]. The adult studies found an association between CD25 expression and adult Ph+ B-ALL cases. However, these studies use different methods to define a positive population, for example, some use an isotype control [13-15]. Moreover, different thresholds for assigning positive and negative CD25 expression were also used [13-15]. Another study did not describe an optimal cutoff [15]. An arbitrary cut off of 20% to define cases as positive for CD25 expression was used by another [14], while yet another used 30% as a cut off as determined by ROC curve [13]. In contrast to these studies, we used the normal lymphocyte population in each individual patient sample to determine positive and negative populations. We also found 25% to be an optimal cutoff as determined by ROC curve to define positive and negative CD25 expression in a pediatric population. We found 3.6% of pediatric B-ALL patients in our institution to be positive for the Philadelphia chromosome translocation by chromosome and FISH analysis, which is consistent with previously published findings of 3-5% prevalence in pediatric B-ALL [9, 10]. Our analysis of pediatric patients diagnosed with B-ALL found significantly higher expression of CD25 in Ph+ B-ALL compared to that of Ph-negative B-ALL. Because of its excellent specificity (97%) and NPV (99%), CD25 could be used as an initial screening test for *BCR-ABL1* in pediatric B-ALL, with the added benefit of a more rapid turnaround time for flow cytometry results than for FISH analysis. However, the low prevalence of *BCR-ABL1* in pediatric B-ALL patients results in a higher number of false positives and a relatively low sensitivity of 75%. With a PPV of only 50% for CD25, potential use of CD25 expression as a diagnostic test for *BCR-ABL1* is limited [18]. Nonetheless, identification of CD25 in a case of B-ALL could suggest prioritization of FISH analysis in order to confirm suspicions for Ph+ B-ALL, thus permitting earlier identification of Ph+ B-ALL and enrollment on appropriate treatment protocols.

We found CD25 expression, using a 25% cutoff, to be a specific and relatively sensitive marker for identifying Ph+ B-ALL in a pediatric population. Further investigation, however, is needed to determine optimal method and cutoff to assign cases as positive or negative for CD25 expression in adult and pediatric B-ALL as well as a prognostic significance of CD25 expression with or without Philadelphia chromosome.

Disclosure of conflict of interest

None.

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