Common genetic abnormalities and phenotypic scoring in Saudi patients with acute B-lymphoblastic leukemia

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ABSTRACT

INTRODUCTION

Flow cytometry (FCM) is considered to be the main factor and is a faster and less expensive technique in the diagnostic and follow-up stages of B-acute lymphoblastic leukemia (B-ALL) patients. Cytogenetic analysis is a critical and significant step in the diagnosis, but sometimes it fails to provide meaningful and sufficient information. This is due to lymphoid neoplasms’ lower mitotic ability in the in-vitro environment. Furthermore, specific abnormalities, such as t(12; 21) (p13; q22), have no discernible effect on chromosome morphology. In this situation, karyotype analysis by cytogenetic should be supported by molecular studies for the identification of fusion genes that resulted from translocations by using fluorescent in situ hybridization (FISH) and real-time polymerase chain reaction (RT-PCR) [1, 2]; cytogenetic analysis also has a crucial role in determining the prognostic significance of B-ALL cases. For example, some cytogenetic abnormalities are associated with poorer outcomes, like the Philadelphia chromosome [t(9; 22)], rearrangements of the intrachromosomal amplification of the AML1 gene (iAMP21) and the MLL gene (chromosome 11q23), while others are associated with good prognosis, such as high hyper-diploidy (HD) (51-65 chromosomes) and the ETV6-RUNX1 fusion [3]. Recent studies have shown that cell phenotype can be used to predict the cells’ cytogenetic abnormalities, which can provide an early tool for the management of high-risk cases of pre-B cell ALL, especially in hospitals with limited laboratory facilities. For example, aberrant expression of myeloid specific cluster of differentiation (CD) markers such as CD66 and CD25 could be an indicator of the presence of specific types of chromosomal aberration known as Philadelphia chromosome t(9; 22) [4]. Expression of CD15 and the absence of CD10 is associated with MLL gene rearrangement, typically t(4; 11) [5]. B-ALL with t(9; 22) sometimes shows expression of CD13 and CD33 [6]. This study was conducted to create a scoring system (SS) by FCM based upon the percentage of expression of multiple CD markers involved in the EuroFlow panel in correlation with a common cytogenetic aberration in Saudi B-ALL patients.

METHODS

Data was obtained from 111 patients diagnosed with B-ALL at King Fahad Specialist Hospital in Dammam (KFSHD) based on FCM and cytogenetic laboratory results. The collected data was from peripheral blood (PB) and bone marrow (BM) samples that were received in the Department of Pathology and Laboratory Medicine at KFSHD from January 2016 to August 2021. The main surface markers included in this study were CD34, CD10, CD66, CD13, CD33, CD123, CD81, CD24, CD20, CD58, CD38, CD9, TdT, CD15, and CD65. The minimum cutoff for PB or BM blast infiltration rate was 20.0%. To classify the case...
as a B-ALL case, cells should express the positivity of two of cytoplasmic CD79a, cytoplasmic CD22, and strong expression of CD10. Myeloperoxidase (MPO) positivity analyzed by FCM should be less than 10.0% to exclude mixed phenotypic acute leukemia (MPAL). All PB or/and BM samples were prepared using EuroFlow staining procedures for the B-ALL diagnostic panel and analyzed using FACS CANTO II instruments. According to EGIL criteria, the threshold for defining the marker as positive on the blast cells for any monoclonal antibody is 20.0%. However, there is an exception for some cytoplasmic markers like MPO, cytoplasmic CD79a, TdT, and cytoplasmic CD3, and these markers are considered positive if their level of expression is 10.0%. Therefore, in this study, the data was retrieved as numerical values to minimize the bias for reporting positive or negative expressions. Chromosomal analysis was performed using FISH and karyotyping procedures for obtaining diagnostic BM or PB samples. For karyotyping, samples were cultured; chromosomes were harvested and then stained by using the G-banding technique. For FISH, all samples were analyzed using reverse transcription-polymerase chain reaction to detect fusions of interest (ETV6/RUNXI fusion, BCR/ABL1 fusion, and TCF3/PBX1 fusion). Descriptive results, including graphical displays, were presented as mean-standard deviation (SD) for all quantitative variables. The percentage has been reported for all qualitative variables. Receiver operating characteristic (ROC) analysis of potential markers in B-ALL has been performed to identify different cytogenetic abnormalities. Sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) have been reported for each proposed FCM SS for predicting the respective cytogenetic category. A p-value of 0.05 (two-tailed) was considered statistically significant. All statistical analyses have been performed using SPSS (statistical package for social sciences version 25.0).

**RESULTS**

After successful cytogenetic karyotyping and FISH analysis, 56 (50.5%) were classified as HD, 20 (18.0%) were ETV6/RUNXI positive cases, 17 (15.3%) were classified as hypodiploidy, 13 (11.7%) were BCR/ABL1 positive cases, and five (4.5%) were diagnosed as TCF3/PBX1 positive cases. The percentages of various antigens in the five different cytogenetic abnormalities were investigated. These antigens were considered the most relevant CD markers in the EuroFlow specific B-ALL antibodies panel, which included: CD34, CD20, CD66, CD58, CD38, CD10, CD33, CD117, CD13, TdT, CD9, CD22, CD24, NG2, CD15+65, CD123, CD81, and CD7. The immunophenotypic criteria, including the percent and intensity of expression of each marker, are specified and determined for each different cytogenetic abnormality. Among the panel marker percentages comparisons, only CD34, CD20, CD9, CD123, CD66, CD13, CD81, and TdT showed low p-values and were considered statistically significant between the different aberrations. Other markers showed high p-values, and there were no significant differences between the groups’ means of their percentages. A ROC curve analysis was performed for all the significant CD markers. Acceptable markers that showed an AUC of 0.7 were CD20, CD9, CD66, CD20, and CD123. These markers were considered useful discriminative markers between the different cytogenetic abnormalities. Other markers were included in this study as ancillary markers and had an AUC almost close to 0.7. These ancillary markers were CD13, CD34, CD81, and TdT (Table 1).

In this cytogenetic group, the common immunophenotypic criteria between the cases were the high expression of CD38, CD10, TdT, CD22, CD24, CD81, and CD38. The intermediate expression was related only to CD123. These cells also show the lowest CD34, CD13, CD33, and CD9. CD20, CD66, NG2, CD15+65, and CD7 are never expressed in this group. Although CD117 positivity was shown in two sporadic cases, there was no consistent expression in the similar cases within this group (Figure 1). Of the different EuroFlow CD markers, only CD9 (95% CI: 21.1-58.5), CD13 (95% CI: 3.5-32.8), CD66, and CD34 (95% CI: 0.0-47.3) were considered as discriminative markers of the ETV6/RUNXI fusion group. According to the comparative expression range of the significant markers, by using the mean, standard error (SE), and SD, we proposed the following: CD66 is never expressed in the ETV6/RUNXI group, so a +2 score will be assigned for its expression. CD9 was included in this SS because of its preferable lower expression in this group. If the expression of CD9 falls between 0 and 47, a +2 score will be given to ETV6/RUNXI. According to our data, CD13 has a consistent expression in ETV6/RUNXI cases. If there is any expression of CD13 between 20.0 and 50.0%, the score will be +1 for this group only. CD34 showed a different lower positivity range than ETV6/RUNXI, so if the blasts showed an expression between 20.0-49.0%, a score of +2 would be given to the ETV6/RUNXI group (Table 1).

**Table 1. Proposed predictive scoring system for ETV6-RUNXI, TCF3-PBX1, BCR-ABL1, HD, and hypodiploidy by using the most predictive markers in the EuroFlow B-ALL panel**

<table>
<thead>
<tr>
<th>CD marker</th>
<th>Percent of expression</th>
<th>TCF3/PBX1</th>
<th>BCR/ABL1</th>
<th>Hyper-diploidy</th>
<th>Hypodiploidy</th>
<th>ETV6/RUNXI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>20.0-79.4%</td>
<td>0</td>
<td>+3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD20</td>
<td>20.0-38.0%</td>
<td>0</td>
<td>0</td>
<td>+2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD66</td>
<td>70.0-91.0%</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD66</td>
<td>40.0-70.0%</td>
<td>0</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD66</td>
<td>20.0-35.0%</td>
<td>0</td>
<td>0</td>
<td>+2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD66</td>
<td>0.0%</td>
<td>+2</td>
<td>0</td>
<td>0</td>
<td>+2</td>
<td>0</td>
</tr>
<tr>
<td>CD9</td>
<td>90.0-100.0%</td>
<td>+1</td>
<td>+1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD9</td>
<td>0.0-47.0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+3</td>
<td>0</td>
</tr>
<tr>
<td>CD123</td>
<td>0.0-19.0%</td>
<td>+3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD13</td>
<td>40.0-70.0%</td>
<td>0</td>
<td>0</td>
<td>+2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD34</td>
<td>80.0-100.0%</td>
<td>0</td>
<td>+2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD34</td>
<td>20.0-63.0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>CD34</td>
<td>0.0%</td>
<td>+3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD81</td>
<td>20.0-70.0%</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TdT</td>
<td>0.0-40.0%</td>
<td>+2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 1. Comparative boxplots of five groups hyper-diploidy, hypoploidy, TCF3-PBX1, ETV6-RUNX1, and BCR-ABL1 regarding percentages of antigens expression of selected EuroFlow B-ALL panel markers: Each antigen expression numerical value is representing a particular case of the total cases of B-ALL (n=111) (Source: Authors’ own elaboration)
Figure 1 (continued). Comparative boxplots of five groups hyper-diploidy, hypoploidy, TCF3-PBX1, ETV6-RUNX1, and BCR-ABL1 regarding percentages of antigens expression of selected EuroFlow B-ALL panel markers: Each antigen expression numerical value is representing a particular case of the total cases of B-ALL (n=111) (Source: Authors’ own elaboration)
This SS application resulted in 15 true positive (TP) cases, five false negative (FN) cases, 89 true negative (TN) cases, two false positive (FP) cases, 75.0% SN, 97.0% SP, 88.0% PPV, and 94.0% NPV. This group IPT is composed of high expressions of CD58, CD38, CD10, CD9, CD22, CD24, and CD81. Low expression of TdT is noticed in this group. CD34, CD20, CD66, CD33, CD117, CD13, NG2, CD15+65, and CD123 are never expressed in this group (Figure 1). Of the different EuroFlow CD markers, only CD9 (95% CI: 92.7-100), CD123, and TdT (95% CI: 0.0-93.5) were shown to be valuable markers for discrimination. According to the comparative expression range of the significant markers by using the mean, SE, and SD, we proposed the following: A +2 score will be given to this group if TdT expression is between 0.0-40.0%. In almost all cases, TCF3-PBX1 had a unique negative expression of CD123. So, the score will be given as a +2 score for CD123 negativity. TCF3-PBX1 showed the highest CD9 expression when compared with other groups, but this characteristic was also found with BCRABL1. So we proposed that if CD9 expression is between 90.0-100%, a +2 score will be offered to TCF3-PBX1 (Table 1).

After the application of the proposed SSs, the result was for TCF3-PBX1 group 4 TP cases, one FN case, 106 true TN cases, 80.0% SN, 100% SP, 100% PPV, and 99.0% NPV. This group has high CD34, CD58, CD38, CD10, TdT, CD9, CD22, and CD24. CD20. This group also had an intermediate expression of CD66 and a low expression of both CD123 and CD81. These cases had no CD33, NG2, CD15+65, or CD7. Although some sporadic cases expressed CD13, there was no consistent expression in the patients of this group (Figure 1).

Four markers of the EuroFlow panel were considered valuable markers for discrimination. These markers were CD20 (95% CI: 20.2-67.7), CD66 (95% CI: 39.1-78.0), CD34 (95% CI: 48.0-91.5), and CD81 (95% CI: 42.0-89.7%). According to the comparative expression range of the significant markers by using the mean, SE, and SD, we proposed the following: if the expression of CD20 is between 28 and 79.4, we can predict that the present cytogenetic abnormality is BCR-ABL1 with a +3
score. If the blasts expressed CD66 between 70.0 and 91.0%, we would give a +1 score for BCR-ABL1. The highest expression of CD34 between the groups was found in BCR-ABL1. If the blasts express CD34 between 80.0 and 100.0%, a +2 score will be added to the BCR-ABL1 group. CD81 was included in this SS due to its unique lower expression in the BCR-ABL1 group when compared to others. If CD81 expression is between 27.0 and 75.0%, a +1 score will be added to the SS (Table 1). Application of the BCR-ABL1 SS resulted in 10 TP, three FN, 98 TN, 76.0% SN, 100% SP, 100% PPV, and 97.0% NPV. The IPT criteria for this group include high expression of CD58, CD10, CD20, CD24, and CD81. CD34, CD38, TdT, CD9, and CD123 have an intermediate expression in this group. These cells never expressed CD117, NG2, CD15+65, and CD7 (Figure 1). In this study, CD20 (95% CI: 4.2-40.6) and CD66 (95% CI: 4.2-40.6) were the only markers that could be used in the SS of the hypoploidy group. They were included in the scoring because of the unique lower expression in this group when compared to others. According to the comparative expression range of the significant markers, by using the mean, SE, and SD, we proposed the following: If the blasts express CD66 between 20.0 and 35.0%, the +2 score will be given to hypoploidy. If CD20 expression is 20-38, we will give a +2 score for this group (Table 1). The hypoploidy SS application resulted in seven TP, 11 FN, and 93 TN, 63.0% SN, 100% SP, 78.0% PPV, and 89.0% NPV. In this group, the immunophenotyping (IPT) was consistent with high expression of CD34, CD58, CD10, CD9, CD22, CD24, CD123, and CD81. Intermediate expression was related to specific markers, including CD66, CD38, and TdT (Figure 1). These cells never express NG2, CD117, CD7, or CD15+65. Even if some sporadic cases show expression of CD20, CD33, and CD13, no consistent expression of this marker was noticed in similar cases included in this group. CD66 (95% CI: 36.4-66.9) was the only marker that was shown to be of value in this group. According to the comparative expression range of the significant markers, by using the mean, SE, and SD, we proposed the following: If CD66 expression is between 40.0 and 67.0%, the +1 score will be given to HD. However, this CD66 expression interval was shared with the other cytogenetic abnormalities (Table 1). The intensity of expression was studied on all EuroFlow markers, and there was no significant correlation between the cytogenetic groups and the intensity of expression. The only two markers that have significant p-values are TdT and CD9. TdT has a significant p-value of 0.001 and was found to be primarily correlated with TCF3-PBX1 because 80.0% of cases show dim or partial expression. This finding was consistent with the diminished expression of TdT in this group. However, partial expression was found in the HD group with 12.5% and hypoploidy with 23.5%, making the intensity not a valuable factor for differentiation. CD9 has a significant p-value, but its expression was bright in all of the cytogenetic aberrations. Partial expression of CD9 was found only in 20.0% of ETV6-RUNX1 cases, which is associated with lower and reduced expression of CD9 in this group. The other cases of ETV6RUNX1 showed a bright expression of CD9 that could not be differentiated from other groups. Therefore, we can conclude that the intensity of expression cannot be included in the proposed SS.

**DISCUSSION**

In B-ALL, several recurrent chromosomal abnormalities have been demonstrated to be prognostic. This study aimed to establish a predictive SS for different common cytogenetic abnormalities in Saudi B-ALL patients. It revealed that the common cytogenetic abnormalities from 2016 to 2021 were ETV6-RUNX1, TCF3-PBX1, BCR-ABL1, hypoploidy, and HD. An SS has been proposed based upon the most significant CD markers within the EuroFlow B-ALL panel. In this research, FCM immunophenotyping of 111 patients diagnosed with B-ALL was used to predict the common Saudi cytogenetic aberration. After a retrospective investigation of the most predictive valuable CD markers, CD20, CD66, CD9, CD123, CD13, CD34, CD81, and TdT were the most valuable markers in discriminating the common cytogenetic abnormalities. CD9, CD123, and TdT were used to predict TCF3-PBX1. According to previous studies, most leukemic cells with TCF3-PBX1 have a characteristic immunophenotype of homogeneous CD19, CD10, and CD9 expression and a total lack of CD34 [7]. The value of CD9 was confirmed and approved by this study since this group showed the highest expression of CD9. CD123 has been reported to be found in most BALL cases, and its overexpression has been correlated with the HD group [8]. Our data revealed a unique negative expression of CD123 in TCF3-PBX1, and it was included in the proposed system. The results obtained by this current study for CD123 expression agree with a study conducted recently. This recent study reported that the diminished or negative expression can be predominantly seen in the TCF3-PBX1 group [9]. TdT expression has not been previously correlated with any specific cytogenetic abnormality. In this study, diminished TdT was related to TCF3-PBX1 compared to the other groups, and it was included in the SS. BCR-ABL1 is most commonly found in elderly patients and is associated with a poor prognosis [9]. These cases in our study were predicted by using CD20, CD66, CD34, and CD81. Expression of CD20 has not been reported to be a significant marker for BCR-ABL1. We were astounded to discover CD20 as a useful marker in the identification of BCR-ABL1 fusions. The CD66 expression was previously discovered to be of value in the same group, which is consistent with our findings [4]. A recent study concluded that CD34 has a high expression in this group [9]. In our study, this group also exhibited the highest levels of CD34 percentage when compared to other groups. The direct correlation of CD81 to BCR-ABL1 fusion has not been studied yet. CD81 was added to the SS because it had the lowest expression intervals between the groups. CD25 was approved previously to be a valuable marker in the detection of BCR-ABL1, but it was not included in the Euroflow fixed panel [10]. ETV6-RUNX1 is the common childhood aberration in B-ALL patients, and it has been reported to have an improved overall survival rate [11]. In the current study, CD9, CD13, CD66, and CD34 were considered the most valuable markers to predict this group. A lower expression of CD9 has been used by a recent study as a predictive marker of ETV6/RUNX1, which is correlated with our finding. When compared to other groups in our study, CD13 expression was specific and consistent with this group. Previous studies also confirmed that ETV6/RUNX1 shows the expression of myeloid antigens [12, 13]. CD34 lower expression and lacking CD66 have been proposed previously and were consistent with our findings [14, 15]. CD27 was reported previously to be a valuable marker in the detection of ETV6-RUNX1, but it was not included in the EuroFlow fixed panel [9]. There are a limited number of studies that have been published to study the phenotype of hypoploidy aberration. It has previously been reported that the hypoploidy group had a phenotype of CD19, cyCD79a, and CD22 without specific expression of aberrant antigens [16]. To our knowledge, CD66
CONCLUSIONS

In this study, four FCM predictive SSs were proposed for the estimation of the most common cytogenetic abnormalities in Saudi B-ALL patients (CD9, CD13, CD66, and CD34) were used to predict ETV6/RUNX1. Using a fixed EuroFlow B-ALL routine acute leukemia panel can be a helpful tool to differentiate between the various cytogenetic aberrations. These proposed SSs showed high SN and SP values, which indicate the need for further evaluation and validation.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES


