Original Article **OPEN ACCESS**

MODESTUM

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

Maha A Alanazi [1] [ID], Faisal M Alzahrani [1] [ID], Saeed Sattar Shaikh [1]* [ID], Amir H Msmar [2] [ID], Fathelrahman Mahdi Hassan [3] [ID]

[1] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, Dammam, SAUDI ARABIA
[2] Deanship of Scientific Research, Imam Abdulrahman Bin Faisal University, Dammam, SAUDI ARABIA
[3] Department of Hematology and Immunohematology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, SUDAN
*Corresponding Author: ssshaikh@iau.edu.sa

ARTICLE INFO	ABSTRACT
	Introduction: B-acute lymphoblastic leukemia (B-ALL) is a malignant neoplasm of hematopoietic stem cells originating in bone marrow and characterized by proliferation of blast cells of lymphoid series. The aim of this study was to predictive scoring system (SS) using a fully standardized EuroFlow eight-color panel. **Methods:** The expression of the different cluster of differentiation (CD) markers involved in the B-ALL EuroFlow panel was investigated by measuring their positivity, percentage, and median fluorescence intensity. **Results:** CD9, CD123, and TdT were used to predict *TCF3PBX1* with 80.0% sensitivity (SN) and 100% specificity (SP). CD20 and CD66 were used to predict hypoploidy with 63.0% SN and 100% SP. As a result, no useful discriminative SS was developed. **Conclusions:** Four SSs were proposed for the prediction of the most common cytogenetic abnormalities of Saudi B-ALL. **Keywords:** flow cytometry, acute lymphoblastic leukemia, immunophenotyping, scoring system

INTRODUCTION

Flowcytometry (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

Table 1. Proposed predictive scoring system for *ETV6-RUNX1, TCF3-PBX1, BCR-ABL1,* HD, and hypoploidy by using the most predictive markers in the EuroFlow B-ALL panel

| CD marker	Percent of expression	TCF3/PBX1	BCR/ABL1	Hyper-diploidy	Hypoploidy	ETV6/RUNX1
CD20	20.0-79.4%	0	+3	0	0	0
CD20	20.0-38.0%	0	0	0	+2	0
CD66	70.0-91.0%	0	+1	0	0	0
CD66	40.0-70.0%	0	0	+1	0	0
CD66	20.0-35.0%	0	0	0	+2	0
CD66	0.0%	+2	0	0	0	+2
CD9	90.0-100%	+1	+1	0	0	0
CD9	0.0-47.0%	0	0	0	0	+3
CD123	0.0-19.0%	+3	0	0	0	0
CD13	40.0-70.0%	0	0	0	0	+2
CD34	80.0-100%	0	+2	0	0	0
CD34	20.0-63.0%	0	0	0	0	+1
CD34	0.0%	+3	0	0	0	0
CD81	27.0-70.0%	0	+1	0	0	0
TdT	0.0-40.0%	+2	0	0	0	0

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/RUNX1* 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).
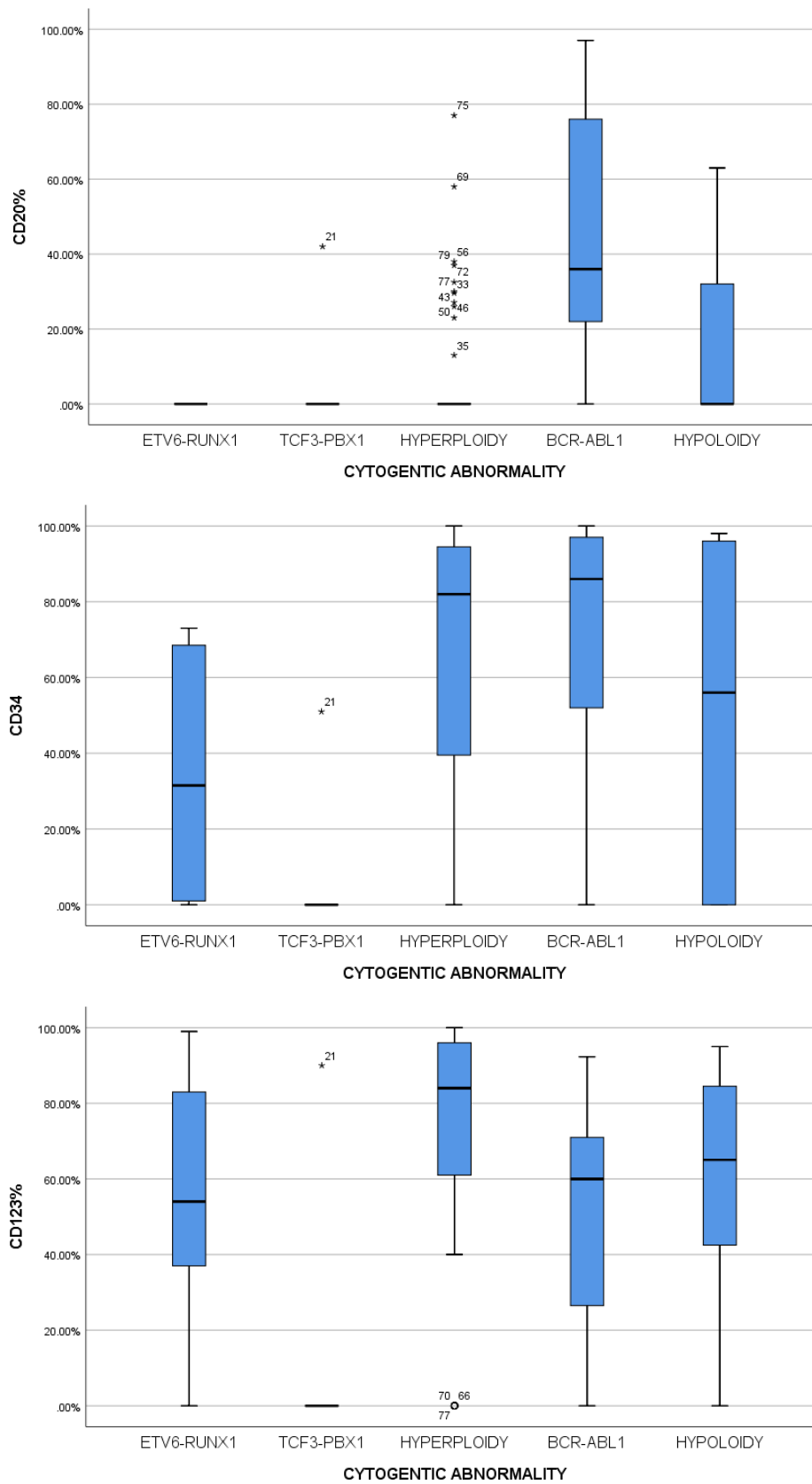
RESULTS

After successful cytogenetic karyotyping and FISH analysis, 56 (50.5%) were classified as HD, 20 (18.0%) were ETV6/RUNX1 positive cases, 17 (15.3%) were classified as hypoploidy, 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**).
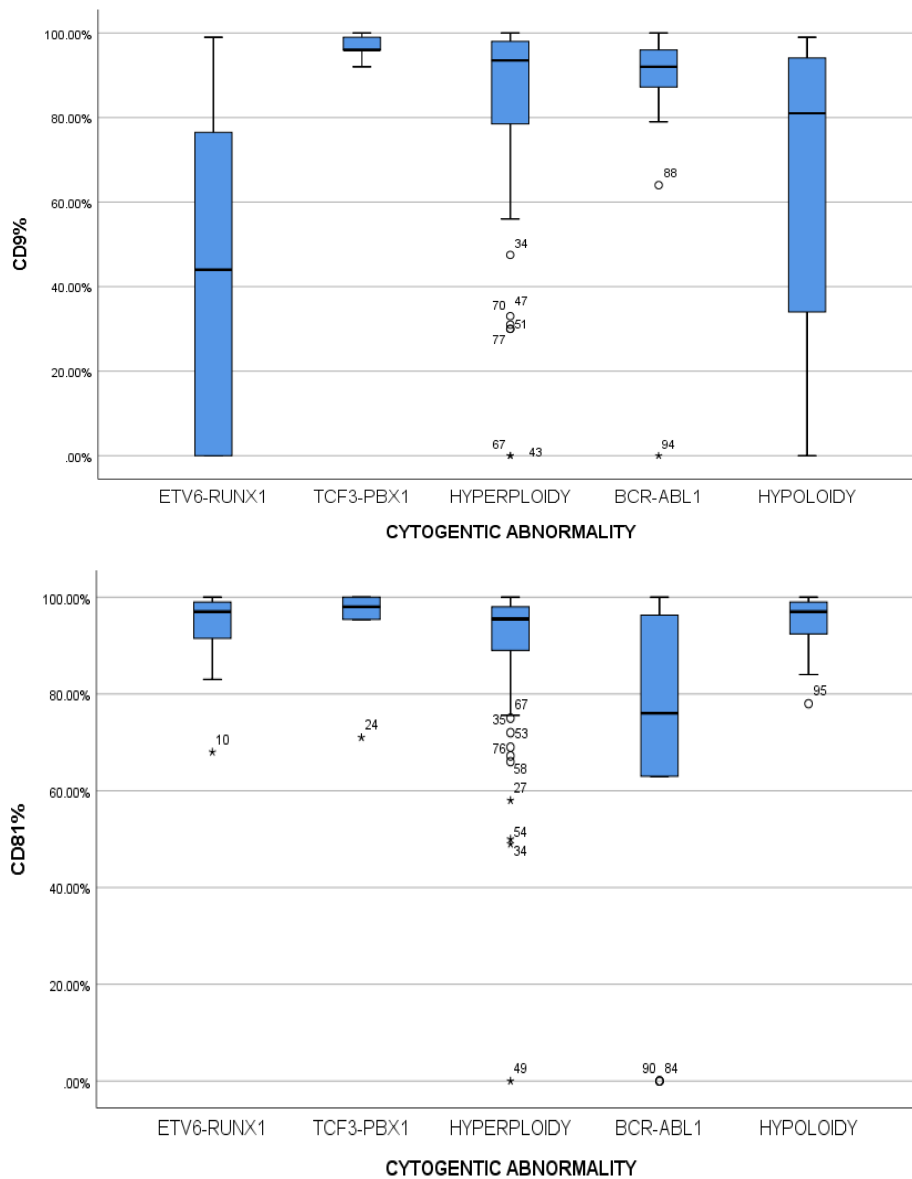
In this cytogenetic group, the common immunophenotypic criteria between the cases were the high expression of CD58, CD10, TdT, CD22, CD24, CD81, and CD38. The intermediate expression was related only to CD123. These cells also show the lowest CD34, CD13, CD33, and CD9. CD20, CD66, NG2, CD15+65, and CD7 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-RUNX1* 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-RUNX1 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 is given to *ETV6RUNX1*. According to our data, CD13 has a consistent expression in *ETV6RUNX1* 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-RUNX1*, so if the blasts showed an expression between 20.0-49.0%, a score of +2 would be given to the *ETV6-RUNX1* group (**Table 1**).
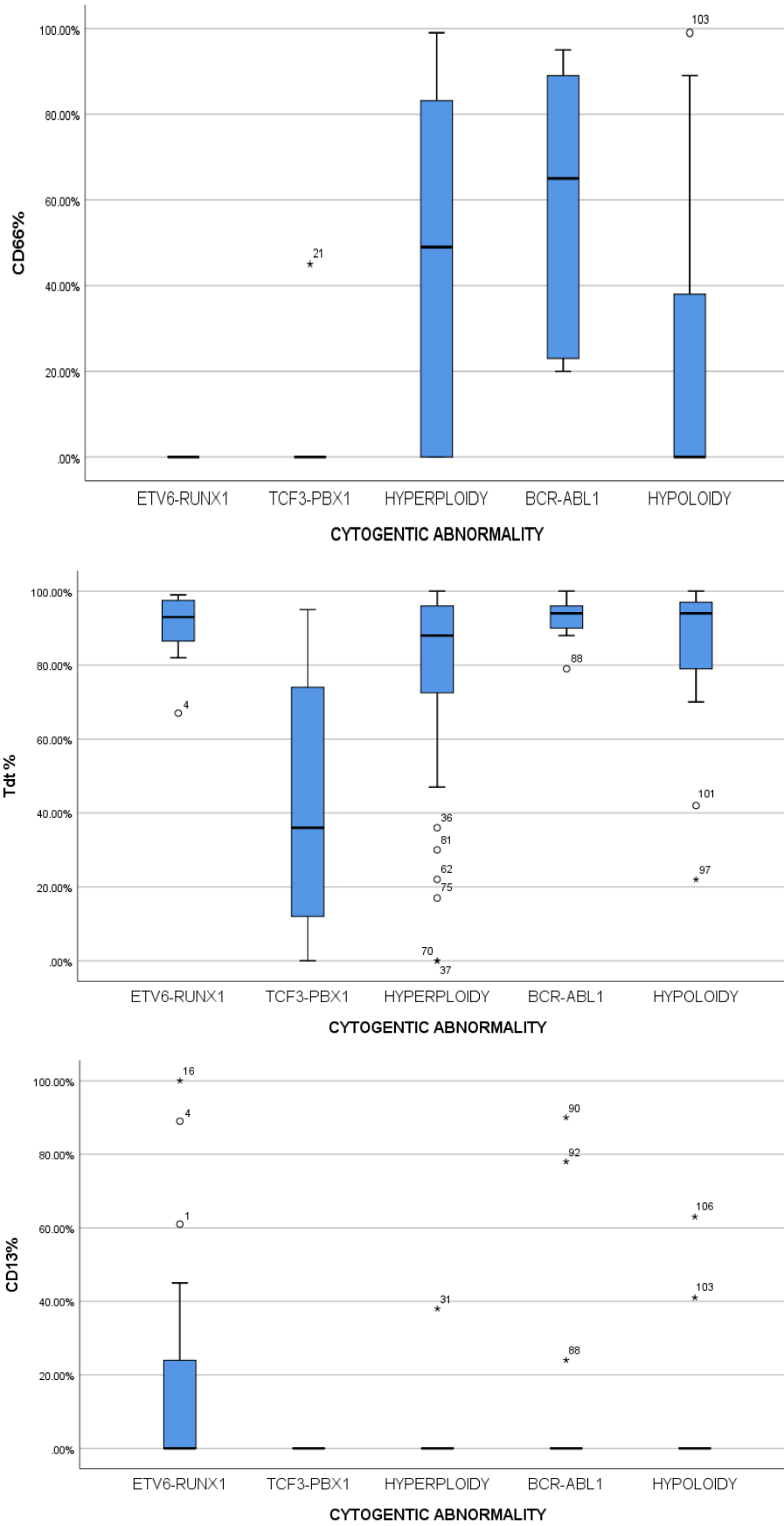
Figure 1. Comparative boxplots of five groups hyper-diploidy, hypoploidy, *TCF3-PBX1*, *ETV6-RUNX1*, and *BCR-ABL1* regarding percentages of antigens expression of selected EuroFlow B-ALL panel markers: Each antigen expression numerical value is representing a particular case of the total cases of B-ALL (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)

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 cells 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%, 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, CD22, 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

is the only aberrant antigen reported to be expressed in hypoploidy [15], while our data revealed that this group had a significantly lower expression of both CD20 and CD66, and they were considered the most valuable markers for this group. CD123 and CD66 have been studied previously as discriminative markers for the HD group [17]. Overexpression of CD123 was recently correlated to HD, but this marker did not prove to have valuable expression in our cohort. Also, our data showed that the HD has an intermediate CD66 expression with overlapped expression intervals with other groups. As a result of that, a valuable discriminative SS for this group could not be established. The immunophenotyping criteria that can be shared with other groups include high expression of CD34, CD58, CD10, CD9, CD22, CD24, and CD81.

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 *ETV6RUNX1*. 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.

REFERENCES

1. Klein U, Tu Y, Stolovitzky GA, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med. 2001;194(11):1625-38. https://doi.org/10.1084/jem. 194.11.1625 PMid:11733577 PMCid:PMC2193527

2. Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. J Clin Invest. 2012;122(10):3407-15. https://doi.org/10.1172/JCI61203 PMid:23023711 PMCid:PMC3461902

3. Hakeem A, Shiekh AA, Bhat GM, Lone AR. Prognostification of ALL by cytogenetics. Indian J of Hematol Blood Transfus. 2014;31(3):322-31. https://doi.org/10.1007/s12288-014-0483-0 PMid:26085716 PMCid:PMC4465518

4. Owaidah TM, Rawas FI, Al Khayatt MF, Elkum NB. Expression of CD66c and CD25 in acute lymphoblastic leukemia as a predictor of the presence of BCR/ABL rearrangement. Hematol Oncol Stem Cell Ther. 2008;1(1):34-7. https://doi.org/10.1016/S1658-3876(08) 50058-6 PMid:20063526

5. Loghavi S, Kutok JL, Jorgensen JL. B-acute lymphoblastic leukemia/lymphoblastic lymphoma. Am J Clin Pathol. 2015;144(3):393-410. https://doi.org/10.1309/AJCPAN7BH 5DNYWZB PMid:26276770

6. Primo D, Tabernero MD, Perez JJ, et al. Genetic heterogeneity of BCR/ABL+ adult B-cell precursor acute lymphoblastic leukemia: Impact on the clinical, biological and immunophenotypical disease characteristics. Leukemia. 2005;19(5):713-20. https://doi.org/10.1038/sj. leu.2403714 PMid:15789066

7. He G-Q, Guo X, Jiang M-Y, et al. Co-occurrence of TCF3-PBX1 gene fusion, and chromosomal aberration in a pediatric pre-B cell acute lymphoblastic leukemia with clitoris swelling. Medicine (Baltimore). 2021;100(8):e24802. https://doi.org/10.1097/MD.0000000000024802 PMid: 33663097 PMCid:PMC7909158

8. Djokic M, Bjorklund E, Blennow E, Mazur J, Soderhall S, Porwit A. Overexpression of CD123 correlates with the hyperdiploid genotype in acute lymphoblastic leukemia. Haematologica. 2009;94(7):1016-9. https://doi.org/10.3324 /haematol.2008.000299 PMid:19454491 PMCid: PMC2704314

9. Tsagarakis NJ, Papadhimitriou SI, Pavlidis D, et al. Flow cytometric predictive scoring systems for common fusions ETV6/RUNX1, BCR/ABL1, TCF3/PBX1 and rearrangements of the KMT2A gene, proposed for the initial cytogenetic approach in cases of B-acute lymphoblastic leukemia. Int J Lab Hematolo. 2019;41(3):364-72. https://doi.org/10.1111/ ijlh.12983 PMid:30730614

10. Tabernero MD, Bortoluci AM, Alaejos I, et al. Adult precursor B-ALL with BCR/ABL gene rearrangements displays a unique immunophenotype based on the pattern of CD10, CD34, CD13 and CD38 expression. Leukemia. 2001;15(3):406-14. https://doi.org/10.1038/sj.leu.2402060 PMid:11237064

11. Hawkins MM, Lancashire ER, Winter DL, et al. The British childhood cancer survivor study: Objectives, methods, population structure, response rates and initial descriptive information. Pediatr Blood Cancer. 2008;50(5):1018-25. https://doi.org/10.1002/pbc.21335 PMid:17849473

12. Blunck CB, Terra-Granado E, Noronha EP, et al. CD9 predicts ETV6-RUNX1 in childhood B-cell precursor acute lymphoblastic leukemia. Hematol Transfus Cell Ther. 2019;41(3):205-11. https://doi.org/10.1016/j.htct.2018.11. 007 PMid:31085145 PMCid:PMC6732401

13. van der Weyden L, Giotopoulos G, Rust AG, et al. Modeling the evolution of ETV6-RUNX1-induced B cell precursor acute lymphoblastic leukemia in mice. Blood. 2011;118(4):1041-51. https://doi.org/10.1182/blood-2011-02-338848 PMid:21628403 PMCid:PMC3622520

14. Pais AP, Amare Kadam PS, Raje GC, et al. RUNX1 aberrations in ETV6/RUNX1-positive and ETV6/RUNX1 negative patients: Its hemato-pathological and prognostic significance in a large cohort (619 Cases) of ALL. Pediatr Hematol Oncol. 2008;25(6):582-97. https://doi.org/10.1080 /08880010802237450 PMid:18728978

15. Kiyokawa N, Iijima K, Tomita O, et al. Significance of CD66c expression in childhood acute lymphoblastic leukemia. Leuk Res. 2014;38(1):42-8. https://doi.org/10.1016/j. leukres.2013.10.008 PMid:24231528

16. Molina O, Bataller A, Thampi N, et al. Near-haploidy and low-hypodiploidy in B-cell acute lymphoblastic leukemia: When less is too much. Cancers (basel). 2021;14(1):32. https://doi.org/10.3390/cancers14010032 PMid:35008193 PMCid:PMC8750410

17. Pierzyna-Świtała M, Sędek Ł, Kulis J, et al. Multicolor flow cytometry immunophenotypingand characterization of aneuploidy in pediatricB-cell precursor acute lymphoblastic leukemia. Cent Eur J Immunol. 2021; 46(3):365-74. https://doi.org/10.5114/ceji.2021.109794 PMid:34764809 PMCid:PMC8574114