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# Positive and Negative Predictive Values of HLA-DR and CD34 in the Diagnosis of Acute Promyelocytic Leukemia and Other Types of Acute Myeloid Leukemia with Recurrent Chromosomal Translocations

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**SUMMARY** The predictive value of HLA-DR and CD34 in the diagnosis of four distinct genetic entities of acute myeloid leukemia (AML) is presently not established. We evaluated the positive and negative predictive values (PPV and NPV, respectively), sensitivity, specificity, and correlation coefficients of HLA-DR and CD34 in AML patients with t(15;17), t(8;21), inv(16), and abn(11q23). In AML with t(15;17) (n = 64), HLA-DR was expressed in 4.68% and CD34 was expressed in 15.62% and none of the cases expressed both HLA-DR and CD34. In AML with t(8;21) (n = 99), HLA-DR, CD34 or both antigens were expressed in the majority of cases (90.90%, 80.80%, and 79.79%, respectively). AML patients with inv(16) (n = 18) and abn(11q23) (n = 31) also highly expressed HLA-DR and CD34. Eight cases of t(8;21) and 1 case of abn(11q23) did not express either antigen. The highest correlation between CD34 and HLA-DR expression values was observed in cases with t(8;21) ($r$ = 0.72) with the lowest correlation in inv(16) ($r$ = 0.035). The PPV and NPV of HLA-DR-negativity plus CD34-negativity to predict t(15;17) was 85% and 100%, respectively, with 100% sensitivity and 92.74% specificity. The PPV and NPV of other myeloid markers such as CD117, MPO and CD11c to diagnose t(15;17) were much lower than those of HLA-DR and CD34. It was concluded that the absence of double negativity of HLA-DR and CD34 strongly predicts against t(15;17). Rare HLA-DR-positive/CD34-negative cases exist in patients with t(15;17) and 8% of t(8;21) cases expressed neither antigen. Further studies should determine whether HLA-DR-positive t(15;17) and HLA-DR-negative/CD34-negative t(8;21) represent a special entity associated with significant prognostic relevance.

Acute myeloid leukemia (AML) is characterized by a clonal expansion of abnormal hematopoietic stem cells and precursors.[1] Various genetic aberrations have been identified in AML patients including structural and numerical chromosomal aberrations, gene mutations, and dysregulated gene expression.[2] The World Health Organization's (WHO) classification recognizes the importance of genetic aberrations in the diagnosis and prognostication of AML and categorizes four unique groups of AML with recurrent chromosomal translocations, *i.e.* AML with t(8;21)(q22;q22), AML with inv(16)(p13q22)/ t(16;16)(p13;q22), AML with t(15;17)(q22;q12), and AML with 11q23 abnormalities into separate entities.[3] These four entities currently constitute about 25-30% of all cases of adult AML.

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Flow cytometric immunophenotyping has become an essential tool for the lineage determination of leukemic "blast" cells that are arrested at a certain differentiation and maturation stages.[4] Various myeloid markers such as CD33, CD13, MPO, CD11c, CD11b, CD117, and CD64 are well recognized and useful for the confirmation of AML and exclusion of acute lymphoblastic leukemia (ALL) and other lymphoid neoplasms.[5-6] CD34, a hematopoietic stem cell marker, is variably expressed in AML and its expression is frequently indicative of the primitive origin of the leukemic cells.[6-7] Another universal antigen, HLA-DR, which belongs to a human leukocyte antigen (HLA) class II, has also been utilized to distinguish a certain type of AML, such as acute promyelocytic leukemia (APL) or AML-M3 from other AML subtypes (M0, M1, M2, M4, M5, M6, and M7)[12,13] as categorized based on the 1976 French-American-British (FAB) Classification.[8-10]

At present, little data exists with respect to the predictive values of HLA-DR and CD34 in the differential diagnosis of AML with recurrent genetic aberrations classified according to the WHO Classification System. In the present study, we aimed to determine the predictive values, sensitivity, and specificity of various immunophenotypic markers, particularly, HLA-DR and CD34, in the diagnosis of AML with t(8;21), t(15;17), inv(16), and 11q23 abnormalities. The correlation coefficients between HLA-DR and CD34 expression values were also derived for each type of AML.

## MATERIALS AND METHODS

### Leukemia samples

This study was a part of a large Leukemia Project approved by the Ethical Committee for Human Research, Faculty of Medicine Siriraj Hospital, Mahidol University. Leukemic samples were obtained from newly diagnosed AML patients who underwent routine hematologic work-ups at the Faculty of Medicine Siriraj Hospital. Conventional chromosome banding studies were performed using standard cytogenetic techniques and chromosomal abnormalities were described according to the International System for Cytogenetic Nomenclature (ISCN).[11]

### Immunophenotypic analysis

Mononuclear cells (MNC) were labeled with the following panel of monoclonal antibodies according to our previously established methods (12-13): CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD33, CD34, CD38, CD41, CD45, CD56, CD57, CD64, CD117, cytoplasmic MPO, TdT, and HLA-DR (BD Biosciences, CA, USA). After 30-minute incubation, cells were lysed in FACS lysing buffer and washed with phosphate-buffered saline (PBS). CD45 and side scatter (SSC) gates were used to select blast windows for multiparameter flow cytometric analysis (FACSCalibur; Becton Dickinson, San Jose, CA, USA). A membrane marker was considered positive when more than 20% of the blast cells expressed it.

### Statistical analysis

Immunophenotypic markers that showed differential expression patterns among the four groups of AML with recurrent chromosomal translocations were identified and a particular marker was selected for the subsequent calculation of the sensitivity, specificity, PPV, and NPV using the following formulas.[14] Sensitivity was derived from the number of truly positive (TP) tests divided by the sum of TP tests and falsely negative (FN) tests while specificity was derived from the number of truly negative (TN) tests divided by the sum of falsely positive (FP) tests and TN tests. PPV was derived from the number of TP tests divided by the sum of TP tests and FP tests while NPV from the number of TN tests divided by the sum of FN tests and TN tests. The gold standard was the presence of specific karyotype according to the WHO classification.

## RESULTS

### Immunophenotypic profiles of AML with recurrent translocations

Two-hundred and twelve AML patients with recurrent chromosomal translocations were identified, including 99 cases with t(8;21), 64 cases with t(15;17), 18 cases with inv(16), and 31 cases with 11q23 abnormalities (abn[11q23]). The majority of cases expressed myeloid antigens, CD13, CD33, and MPO, as expected. MPO was highly expressed in more than 90% of cases with t(15;17) and t(8;21), 88% of cases with inv(16), and half of abn(11q23) cases. CD117 was expressed in about 70% of cases with t(15;17), t(8;21), and inv(16), and half of the

cases with abn(11q23). CD14 was significantly expressed in AML with inv(16) as compared to other groups ($p < 0.01$), confirming the myelomonocytic origin of leukemic cells with inv(16). The representative flow cytometric profiles of AML with four different types of translocations are shown in Fig. 1A-D. The similar high SSC profiles can be seen in cases with t(8;21) and t(15;17).

In AML with t(15;17) (n = 64), HLA-DR was expressed in 4.68% and CD34 was expressed in 15.62% of cases, and none of the cases expressed both HLA-DR and CD34 (Table 1). While only three cases of t(15;17) expressed HLA-DR, a slightly higher proportion (10 cases) expressed CD34. Among AML with t(8;21) (n = 99), HLA-DR, CD34 or both antigens were expressed in the majority of cases (90, 80, and 79 cases, respectively). All 18 cases of inv(16) and 30 out of 31 cases of abn(11q23) expressed HLA-DR while 17 cases of inv(16) and 19 cases of abn(11q23) expressed both CD34 and HLA-DR.

**Diagnostic values of HLA-DR and CD34 in the subclassification of AML**

The PPV, NPV and sensitivity, and specificity of CD34 and HLA-DR in the diagnosis of t(15;17), t(8;21), inv(16) and abn(11q23) are summarized in Table 2. CD34-negativity and HLA-DR-

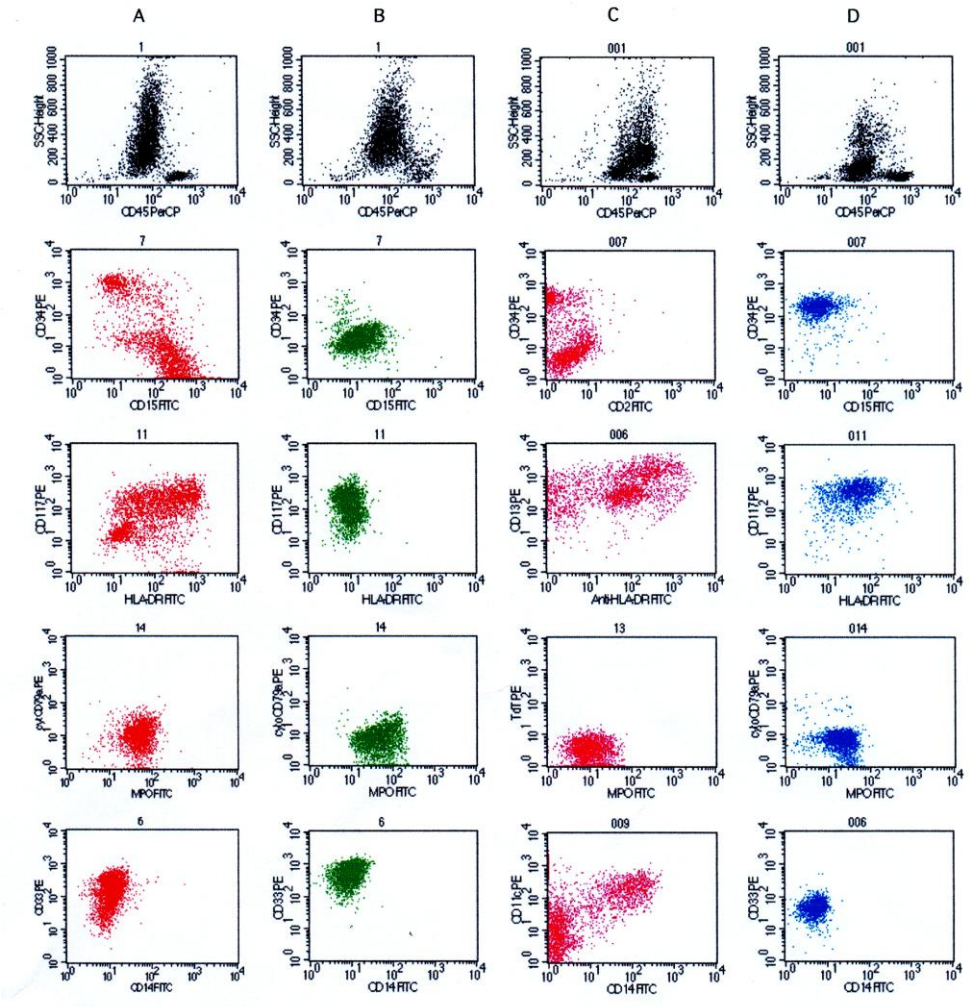


**Fig. 1** Representative flow cytometric profiles of AML with t(8;21) (A), APL with t(15;17)(B), AML with inv(16) (C) and AML with abn (11q23) (D).

negativity had the highest PPV (85%) and the highest NPV (100%) for differentiating t(15;17) from t(8;21), inv(16), and abn(11q23) cases combined. Low PPV and NPV of the dual absence of HLA-DR and CD34 were observed in cases with t(8;21), inv(16), and abn(11q23) when compared to t(15;17). CD34-negativity and HLA-DR-negativity had the highest sensitivity (100%) for differentiating t(15;17) from t(8;21), inv(16) and abn(11q23) as well as the highest specificity (90.8%).

**Correlation of HLA-DR and CD34 in AML with recurrent translocations**

The expression values of HLA-DR and CD34 expression in each AML patient were plotted and the correlation coefficients were derived for each AML subtype (Fig. 2). The best correlation was seen in t(8;21), as shown in Fig. 2A, with a correlation coefficient value of 0.72 ($p$ = 0.00) followed by abn(11q23) ($r$ = 0.368, $p$ = 0.042), t(15;17) ($r$ = 0.134, $p$ = 0.290), and inv(16) ($r$ = 0.035, $p$ = 892). The majority of cases with t(15;17) had HLA-DR and CD34 expression values below 20% with very few cases having HLA-DR values over 20% (Fig. 2B). In inv(16) cases, most cases had high HLA-DR expression although variable CD34 expression values were observed (Fig. 2C). In patients with 11q23 abnormalities, very high HLA-DR and CD34 values were observed in the majority of cases. However, 11 cases of abn(11q23) patients had a negative CD34 expression value (< 20%) despite a very high HLA-DR value (Fig. 2D). Eight cases of t(8;21) and 1 case of abn(11q23) did not express either antigen.

**Comparison of various different markers to differentiate t(15;17) from t(8;21)**

Because of the similar CD45/SSC profiles between t(8;21) and t(15;17) as shown in Fig. 1A-B, we also explored the diagnostic values of various myeloid markers routinely used in flow cytometric immunophenotyping. The PPV, NPV, sensitivity, and specificity of CD117, CD33, CD13, MPO, and CD11c for the diagnosis of t(15;17) are demonstrated in Table 3. CD33 and CD13 had the highest sensitivity (100%) in screening for t(15;17) but their specificity was very low (3-4%) to distinguish between t(15;17) and t(8;21). MPO had the second highest sensitivity (94.44%) associated with very low specificity. CD117 had moderate sensitivity but very low specificity, PPV, and NPV. CD11c had the lowest sensitivity, PPV, and NPV as compared to other myeloid markers. The highest NPV was observed for CD33 and CD13. All myeloid markers shown in Table 3 did not have a good PPV nor adequate specificity to assign AML cases with t(15;17).

## DISCUSSION

Flow cytometry is a valuable tool for the diagnosis of acute leukemia and is now replacing cytochemistry in most routine laboratories worldwide.[4-6] This study evaluated the utilization of flow cytometric immunophenotyping in the differential diagnosis of AML cases genetically classified according to the

**Table 1** CD34 and HLA-DR expression in AML with different types of translocations

| Antigen | t(15;17) | | t(8;21) | | inv(16) | | abn(11q23) | |
|---|---|---|---|---|---|---|---|---|
| | No. of positive cases/total cases | % positive cases | No. of positive cases/total cases | % positive cases | No. of positive cases/total cases | % positive cases | No. of positive cases/total cases | % positive cases |
| **CD34+** | 10/64 | 15.62 | 80/99 | 80.8 | 17/18 | 94.44 | 19/31 | 61.29 |
| **HLA-DR+** | 3/64 | 4.68 | 90/99 | 90.9 | 18/18 | 100 | 30/31 | 96.77 |
| **CD34+ HLA-DR+** | 0/64 | 0.00 | 79/99 | 79.79 | 17/18 | 94.44 | 19/31 | 61.29 |
| **CD34- HLA-DR+** | 3/64 | 4.68 | 11/99 | 11.11 | 1/18 | 5.55 | 11/31 | 35.48 |
| **CD34+ HLA-DR-** | 10/64 | 15.68 | 1/99 | 1.01 | 0/17 | 0.00 | 0/31 | 0.00 |
| **CD34- HLA-DR-** | 51/64 | 79.68 | 8/99 | 8.08 | 0/19 | 0.00 | 1/31 | 3.22 |

**Table 2** Values of CD34 and HLA-DR in the diagnosis of AML with t(15;17) from AML with other translocations

| **Diagnostic values of CD34 and HLA-DR in the differentiation of t(15;17) AML from t(8;21) AML** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **t(15;17) AML** (n = 64) | | | | **t(8;21) AML** (n = 99) | | | |
| **Immunophenotype** | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) |
| **CD34- and HLA-DR-** | 86.44 | 100 | 100 | 90.80 | 13.55 | 0 | 9.19 | 0 |
| **CD34+ and HLA-DR+** | 0 | 13.5 | 0 | 9.19 | 100 | 86.44 | 90.80 | 100 |
| **Diagnostic values of CD34 and HLA-DR in the differentiation of t(15;17) AML from inv(16) AML** | | | | | | | | |
| | **t(15;17) AML** (n = 64) | | | | **inv(16) AML** (n = 19) | | | |
| **Immunophenotype** | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) |
| **CD34- and HLA-DR-** | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 |
| **CD34+ and HLA-DR+** | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 |
| **Diagnostic values of CD34 and HLA-DR in the differentiation of t(15;17) AML from abn(11q23) AML** | | | | | | | | |
| | **t(15;17) AML** (n = 64) | | | | **abn(11q23) AML** (n = 31) | | | |
| **Immunophenotype** | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) |
| **CD34- and HLA-DR-** | 98.07 | 100 | 100 | 95.0 | 1.92 | 0 | 5.0 | 0 |
| **CD34+ and HLA-DR+** | 0 | 1.92 | 0 | 5.0 | 100 | 98.07 | 95.0 | 100 |
| **Diagnostic values of CD34 and HLA-DR in the differentiation of AML with t(15;17) from other AML** | | | | | | | | |
| | **t(15;17) AML** (n = 64) | | | | **t(8;21), inv(16) and abn(11q23) AML** (n = 149) | | | |
| **Immunophenotype** | **PPV** (%) | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) | **PPV (%)** | **NPV** (%) | **Sensitivity** (%) | **Specificity** (%) |
| **CD34- and HLA-DR-** | 85.0 | 100 | 100 | 92.74 | 15.0 | 0 | 7.25 | 0 |
| **CD34+ and HLA-DR+** | 0 | 15.0 | 0 | 7.25 | 100 | 85.0 | 92.74 | 100 |

WHO Classification. Four groups of patients with distinct chromosomal translocations were identified and their immunophenotypic markers were compared. We particularly focused on HLA-DR and CD34 expression as previous data are available only for the diagnostic values of HLA-DR and CD34 in the classification of AML according to the traditional FAB co-operative group's morphology-based criteria.

HLA-DR is a known marker that is associated with early precursor cells and actively metabolizing cells. The majority of AML cases (80%) and precursor-B ALL cases (97%) expressed HLA-DR.[7,15] Our previous study of 262 patients revealed that the majority of AML cases expressed HLA-DR with only 18% of AML cases negative for HLA-DR and most of them belonged to FAB-M3 subtype.[12] The present study found that HLA-DR was predominantly absent in almost all cases of t(15;17) as expected due to the known association of APL with t(15;17). However, some of our patients without t(15;17) lacked HLA-DR expression including 9 cases with t(8;21) and one case with abn(11q23). The absence of HLA-DR in non-APL cases has previously been reported. Lazarchick *et al*. pointed out in a

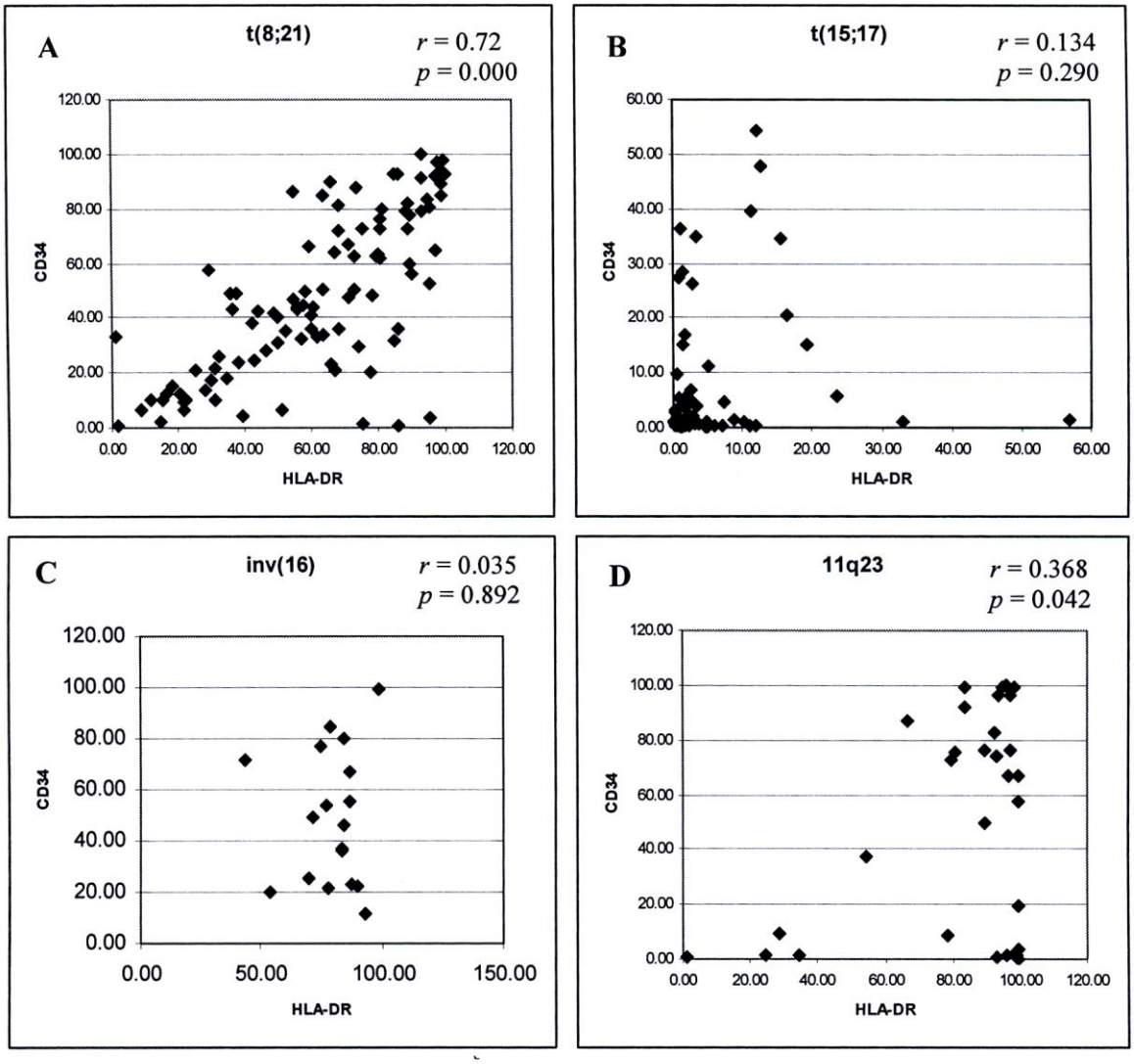


**Fig. 2** Correlation of HLA-DR and CD34 expression values in AML with t(8;21) (A), APL with t(15;17) (B), AML with inv(16) (C) and AML with abn(11q23) (D); each dot represents a single patient and the value on each axis represents percentage of cells expressing a particular marker.

small study of 36 AML patients that cases other than APL, particularly FAB-M2, were also found lacking HLA-DR expression.[16] In a larger study of 248 AML cases, HLA-DR-negative cases were identified in 43 patients, with only half of the cases belonging to APL.[15] None of the HLA-DR-negative non-APL cases had t(8;21), t(15;17), or inv(16) by cytogenetic analysis but 17 of them (74%) were labeled as M1/M2 and 5 of them as M4/M5 (22%) by morphology.[15] Seventeen out of 124 cases of FAB-M1/M2 were also HLA-DR-negative.[7] HLA-DR negativity has thus been observed in some non-APL cases and may not be adequate by itself to confirm the presence of APL with t(15;17).

The expression of CD34 has been observed in 60% of AML cases with varying degree of expression in various FAB subtypes.[7,12,15] FAB-M0-M1 highly expressed CD34 as compared to other subtypes.[17] A lower frequency of CD34 expression has been reported in leukemic cells of a monocytic lineage such as M4/M5 as well as promyelocytic lineage (M3).[7] The present study found that CD34 was significantly correlated with HLA-DR in cases with t(8;21). However, the other three types of translocations did not have such a high correlation, reflecting different effects of karyotype on the immunophenotype of leukemic cells. Three cases with t(15;17) had a high HLA-DR expression but their CD34 values were contradictorily low (<20%). On the other hand, ten cases of t(15;17) with negative HLA-DR demonstrated quite a variable, yet positive, CD34 expression value, ranging from 20% to 55%. The low correlation of CD34 and HLA-DR in the non-t(8;21) cases may reflect the different differentiation and maturation stages of cells affected by the translocations as well as the composition of cells within each particular type of leukemia. A highly variable CD34 expression in cases with inv(16) may correspond to the relatively variable number of monocytic cells and myeloblastic cells in the patients. Very high HLA-DR and CD34 values as observed in the majority of cases with 11q23 abnormalities could reflect the primitive origin of this type of leukemia.

Although the association between HLA-DR and CD34 expression and APL has been described, the specificity and the predictive values of both markers in the diagnosis of each type of AML with recurrent cytogenetic abnormalities were rarely calculated and reported. [18-19] Khoury *et al*. revealed that the absence of $CD34^+$, $HLA\text{-}DR^+$ or $MPO^+$ would preclude t(8;21) while the expression of $CD34^+/CD19^+/CD56^+$ was highly predictive and could serve as a screening criteria for the t(8;21).[20] Kaleem *et al*. reported the PPV of HLA-DR and CD34 double negativity of 72% for morphologically classified APL cases which was slightly lower than our value of 85% in cytogenetically confirmed cases with t(15;17).[7] It is not clear if all APL cases in the mentioned study was confirmed at a cytogenetic level to have t(15;17). Interestingly, eight percent of our t(8;21) cases lacked both HLA-DR and CD34 expression. A comparable percentage was also reported in one study whereby 13/124 cases (10%) of M1/M2 did not express either antigen.[7] A recent study from Japan suggests that HLA-DR negativity in M1/M2 cases may be associated with *FLT3* and *NPM1* mutations.[20-21] Larger clinical studies are needed to confirm whether HLA-DR-negative M1/M2 represents a unique entity. With respect to the prognostic relevance of CD34 and HLA-DR, both markers were shown to be predictors of failure to achieve complete remission and poor clinical out-

**Table 3** Comparison of various different markers to assign AML with t(15;17)

| Marker | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| **CD117** | 73.68 | 20.28 | 33.73 | 58.33 |
| **CD33** | 100 | 4.08 | 40.50 | 100 |
| **CD13** | 100 | 3.09 | 39.35 | 100 |
| **MPO** | 94.44 | 4.81 | 39.23 | 57.14 |
| **CD11c** | 10.34 | 12.19 | 7.69 | 16.12 |

come in AML cases.[22-24] CD34-positive patients had a lower CR rate, overall survival and disease-free survival than those in the CD34-negative group.[22-24] Lower remission rate was also reported in HLA-DR positive APL patients.[23]

In conclusion this present study established the predictive values of HLA-DR and CD34 in the diagnosis of AML with distinct genetic entities. The calculated PPV, NPV, sensitivity, and specificity reveals the important value of HLA-DR and CD34 in the differential diagnosis of t(15;17) from other recurrent translocations. HLA-DR and CD34 expression levels correlated well in the cases with t(8;21) whereas CD34 expression level was found to be more heterogeneous with respect to HLA-DR in the cases with t(15;17), inv(16), and abn(11q23), possibly reflecting differential impact of karyotype on the immunophenotype of leukemic cells. Rare HLA-DR-positive/CD34-negative cases exist in patients with t(15;17) and 8% of t(8;21) cases expressed neither antigen. Further studies should determine whether HLA-DR-positive t(15;17) and HLA-DR-negative/CD34-negative t(8;21) represent a special entity associated with significant prognostic relevance.

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