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The diagnostic power of CD117, CD13, CD56, CD64, and MPO in rapid screening acute promyelocytic leukemia

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Research note

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Abstract

Objective

The analogous immunophenotype between HLA-DR-negative acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL) causes APL rapid screening to become difficult. This study aimed to identify the associated antigens for APL and the best model in clinical uses.

Results

A total of 36 APL (*PML-RARA*+) and 29 HLA-DR-negative non-APL patients were selected for this study. When a cut-off point of 20% events was applied to define positive or negative status, APL and non-APL patients share a similar immunophenotype of CD117, CD34, CD11b, CD13, CD33, and MPO ($P > 0.05$). However, expression intensity of CD117 ($P = 0.002$), CD13 ($P < 0.001$), CD35 ($P < 0.001$), CD64 ($P < 0.001$), and MPO ($P < 0.001$) in APL are significantly higher while CD56 ($P = 0.049$) is lower than in non-APL subjects. The Bayesian Model Averaging (BMA) analysis identified CD117 (≥ 49% events), CD13 (≥ 88% events), CD56 (≤ 25% events), CD64 (≥ 42% events), and MPO (≥ 97% events) antigens as an optimal model for APL diagnosis. A combination of these factors resulted in an area under curve (AUC) value of 0.98 together with 91.7% sensitivity and 93.1% specificity, which is higher than individual values (AUC were 0.76, 0.84, 0.65, 0.82, and 0.85, respectively) ($P = 0.001$).

Introduction

Acute promyelocytic leukemia is a hematological malignancy which is characterized by a translocation between chromosome 15 (promyelocytic leukemia gene, *PML*) and chromosome 17 (retinoic acid receptor alpha gene, *RARA*), the t(15;17)(q22;q11) translocation, leading to the formation of *PML-RARA* fusions in hematopoietic stem cells [1–3]. These fusions can be detected in > 95% APL patients with three major transcript subtypes (bcr1, bcr2, and bcr3) depend on the breakpoints of *PML* gene, and some rare subtypes [3].

Regarding the *PML-RARA* fusions, all-*trans* retinoic acid (ATRA) and arsenic trioxide are highly effective agents that are combined in a current treatment method for APL patients [4–7]. According to the recommendations of European LeukemiaNet, treatment with ATRA should be initiated immediately to prevent the risk of severe bleeding while a rapid confirmation of *PML-RARA* fusions is mandatory in all cases [8]. This diagnostic test was recommended to perform on bone marrow cells, by the fluorescence in situ hybridization (FISH) and real-time quantitative polymerase chain reaction (RQ-PCR) methods [8]. Of which, PCR was used as the gold standard method for over 10 years [9]. Besides, the immunostaining with anti-PML antibodies can be used to surrogate for genetic testing. However, this method requires an experienced examiner to do while results are less reproducible [8].

Some immunophenotypic markers as CD34, CD117, HLA-DR, CD13, CD9, CD18, CD2, and CD11a, CD11b might be helpful to guide the APL diagnosis in a fasting method with turnaround time just in two hours [10]. Previous studies have shown that combination some these antigens help to detect APL with high accuracy, sensitivity, and specificity [11–16]. In reality, the morphology and immunophenotype of HLA-DR-positive AML are different from APL while an analogous immunophenotype can be found in certain cases of HLA-DR-negative AML [17–19]. This causes APL screening to become much more difficult in clinically. All of the above studies investigated the diagnostic values of flowcytometric antigens which used a control group containing > 50% HLA-DR-positive AML patients [11–16]. A few studies mention the role of these markers in comparison with a similar phenotype control group [20, 21]. We compared the antigen expression level between APL and HLA-DR-negative non-APL patients and identified the associated markers with APL together with an optimal model in clinical diagnostics.

Materials And Methods

Patients

A total of 65 newly diagnosed AML patients with HLA-DR-negative were selected for this study at Cho Ray hospital from Feb-2016 to March-2020 (approval number 602-BVCR-HDDD) (Additional file 1: Figure S1). Because of a retrospective study, patients were not required to write consent forms. Among them, 36 cases were confirmed APL by the presence of t(15:17) translocation (median 72.1% investigated myeloid cells) and *PML-RARA* fusions (median 82.9% total transcripts) (Additional file 2: Table S1). Twenty-nine remaining cases with *PML-RARA* negative results were classified into the non-APL group. APL patients had been diagnosed at a median age of 46 years old, and with the white blood cell number of 8.7×10^9/L which were lower than in non-APL patients.

Flowcytometric analysis

In the flowcytometric analysis, a procedure with antibody-panel was performed according to the recommendations of EuroFlow [22]. Briefly, 100 µl bone marrow cells were incubated with a cocktail of antibodies for 15 minutes, and then with 500 µl FACS lysing solution (BD Biosciences). Centrifugation at 3000 rpm for 3 minutes was applied to remove supernatant and debris. Afterward, the samples were washed with 2 ml phosphate-buffered saline solution and re-suspended in 500 µl Sheat solution before acquiring on the 8-colors FACSCanto-II system (BD Biosciences, San Jose CA, USA). A percentage of myeloid cells (Mye.C) positive with each antigen was reported as in (Additional file 3: Figure S2). AML with HLA-DR-negative was distinguished according to the classification criteria of EuroFlow and European LeukemiaNet [22, 23].

Molecular and cytogenetic analyses

The t(15;17) translocation was detected in the bone marrow cells by the FISH technique using kit Vysis LSI PML/RARA Dual Color, Dual Fusion Translocation Probe (Cat No. 01N36-020, Abbott Molecular, Illinois, USA) according to the manufacturer’s instructions. Briefly, the mononucleated cells were collected

and treated with KCl 0.075M solution at 37°C/40 minutes and Carnoy's fixative solution (Abbott Molecular) for 20 minutes. After that, the cells were dropped on a positively charged slide and incubated with a 10 µl probe mixture at 75°C/3 minutes, and 37°C/16–20 hours. Finally, the slide was washed with SSC-NP-40 solution and stained with DAPI-II solution before analyzing by the BioView system (Abbott Molecular). The translocation signals were reviewed and calculated in ≥ 400 cells (Additional file 4: Figure S3).

For the *PML-RARA* transcripts detection, total RNA was extracted from bone marrow cells by using kit QIAamp RNA Blood Mini (Cat No. 52304, Qiagen, Hilden, Germany). The *PML-RARA* transcripts (bcr1, bcr2, and bcr3) were detected by the RQ-PCR technique using kit Ipsogen PML-RARA (Cat No. 672123, 672213, and 672313, Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR reactions were performed and analyzed by the RotorGene Q 5Plex HRM platform (Qiagen, Hilden, Germany). Transcript results were reported as normalized to control gene (*ABL*) copy number (Additional file 4: Figure S3).

Statistical analysis

The Chi-square or Fisher's exact (frequency < 5) tests were used to compare the frequencies while the Kruskal-Wallis rank test was used to compare the expression level of each antigen between groups. To identify the associated markers with APL and optimal model in diagnostics, the BMA statistic was used. The logistic regression was used to construct the receiver operating characteristic (ROC) curve and define the cut-off point together with sensitivity, specificity, and the value under the ROC curve (area under the curve: AUC) of each antigen and optimal model in diagnosis APL. All data analyses were done by R statistical software v.3.5.1 (R foundation, 1020 Vienna, Austria). $P < 0.05$ was considered statistically significant.

Results

Antigen expression between groups

All of 65 cases were negative with HLA-DR antigen, lymphocyte lineage (CD10, cyCD3, cyCD79a, TdT, CD3, CD5, CD7, CD8, CD19, CD20, CD22) and other markers (CD71, CD105, CD16, CD36, IREM2). Among them, data of CD11b and CD35 antigens are available only in 35 and 36 cases, respectively (Additional file 5: Table S2). When a cut-off value of 20% events was applied to define positive or negative status, most of the patients are negative with CD34 (90.8%), CD11b (88.6%), CD14 (98.5%), and CD56 (78.5%) while positive with CD117 (95.4%), CD13 (98.5%), CD33 (100%), CD64 (72.3%), and MPO (myeloperoxidase, 95.4%) (Additional file 5: Table S2). We also noted that APL patients share a similar immunophenotype of CD117 (P=0.418), CD34 (P=0.445), CD11b (P=0.238), CD13 (P=0.446), CD14 (P=0.554), CD33 (positive in 100% cases), and MPO (P=0.084) with non-APL patients. However, when the expression intensity of antigens was shown, we found that median level of CD117 (P=0.002), CD13 (P<0.001), CD35 (P<0.001), CD64 (P<0.001), and MPO (P<0.001) in APL patients are significantly higher

than in non-APL subjects (Figure 1). Contrariwise, the CD56 expression level in APL patients is lower than in others (P=0.049).

Associated factors with APL and optimal model in diagnostics

The BMA analysis has identified five factors including CD117, CD13, CD56, CD64, and MPO which are closely associated with APL (Additional file 6: Figure S4). The probability that these antigens linked to APL were 77%, 100%, 59%, 81%, and 96%, respectively. These factors are also included in the optimal model for APL diagnosis by the BMA analysis. Because of insufficient data, CD11b and CD35 were not included in this analysis.

In the univariable logistic regression, APL was differentiated from non-APL with an accuracy of 76% by CD117 (AUC=0.76; cut-off: ≥49% cells), 84% by CD13 (AUC=0.84; cut-off: ≥88% cells), 65% by CD56 (AUC=0.65; cut-off: ≤25% cells), 82% by CD64 (AUC=0.82; cut-off: ≥42% cells), and 85% by MPO (AUC=0.85; cut-off: ≥97% cells) (Table 1). The multivariable analysis showed that the combination of these factors resulted in a significantly increased accuracy value (AUC=0.98, 95%CI: 0.95–1.00, P=0.001) (Figure 2). The sensitivity and specificity of the optimal model in diagnosis APL were 91.7% (95%CI: 80.6–100.0) and 93.1% (95%CI: 82.8–100.0), respectively.

Discussion

Flowcytometry is a key method that is widely used in the classification of AML and other hematological diseases. An advantage of this method is to give results within two hours which is suitable to use in fast screening of APL to minimize the risk of death caused by the disease. This approach based on the distinct profile of cell antigens between APL and other types of AML [24–27]. Most of the myeloblasts in AML express CD34, HLA-DR, CD117, CD13, CD33, and MPO antigens. Whereas, CD34, HLA-DR, and CD11b antigens are rarely expressed by promyelocytes and myelocytes in APL [24–26]. Thus, the expression of these antigens by APL cells makes difficulties in APL diagnosis. Previous studies have shown the high diagnostic values of cell antigens for APL but with a comparison to an AML control group containing a high percentage of HLA-DR-positive subjects [11–16].

In this study, we compared the antigen profile of APL patients to HLA-DR-negative non-APL subjects and observed no differences of CD117, CD34, CD11b, CD13, CD14, CD33, and MPO status between groups ($P > 0.05$). Nevertheless, expression intensity data of CD117, CD13, CD35, CD56, CD64, and MPO in APL are significantly different from non-APL patients ($P < 0.05$). By the BMA statistics, we identified an optimal model for APL including five impacted markers: CD117, CD13, CD56, CD64, and MPO. Notably, this model can help to differentiate APL from HLA-DR-negative non-APL with 98% accuracy, 91% sensitivity, and 93% specificity (Fig. 2). These are comparable with the results of recent studies [20, 21]. In a multi-center study validated on 205 APL and 629 HLA-DR-negative non-APL AML, Liu et al. demonstrated that two antigen panels, CD64 + CD13 + CD33 + MPO + CD11c- and CD64 + CD13 + CD33 + MPO + CD11c + CD10-CD117 + SSC^{high} (side scatter) help to distinguish APL with nearly 100% sensitivity, specificity, and accuracy [20].

Whereas, Mosleh et al. reported a six-panel marker (HLA-DR-CD11b-CD34-CD64 + CD9 + CD117-/+) in diagnosis APL with diagnostic values of 98% [21]. Interestingly, the authors noted that the uses of CD64 and MPO antigens which are included in our model help to increase accuracy, sensitivity, and specificity in diagnosis [20, 21]. Liu et al. even suggested excluding APL when CD64 is absent [20]. In our study, when CD64 and MPO were left out from the optimal model, the accuracy decreased from 0.98 to 0.92 ($P = 0.025$).

Conclusion

An optimal model including CD117, CD13, CD56, CD64, and MPO might help to differentiate APL from HLA-DR-negative AML with high diagnostic values even in a similar immunophenotype. The prompt diagnosis is also speeded up by the use of multi-colors devices and optimized panel according to EuroFlow recommendations. Of course, the confirmation of *PML-RARA* fusions by RQ-PCR analysis as above is still required and final.

Limitations

In this study, the BMA statistics was used to choose the best markers in diagnosis. However, the sample size of the study is limited while this is a single-center retrospective study. A further prospective study is required to confirm this finding, of which cell antigens as CD11b and CD35 should be collected adequately for the examination.

Abbreviations

APL: Acute promyelocytic leukemia; AML: Acute myeloid leukemia; ATRA: All-*trans* retinoic acid; ABL: Abelson; AUC: Area under the curve; BMA: Bayesian model averaging; CD: Cluster of differentiation; FISH: Fluorescence in situ hybridizations; HLA-DR: Human leukocyte antigen DR isotype; IREM2: Immune receptor expressed in monocytic derived cells; MPO: Myeloperoxidase; PML: Promyelocytic leukemia; RARA: Retinoic acid receptor alpha; RQ-PCR: Real-time quantitative polymerase chain reaction; RNA: Ribonucleic acid; ROC: Receiver operating characteristic.

Declarations

Ethics approval and consent to participate

This study was considered and approved by the Ethics Committees of Cho Ray Hospital (approval number: 602-BVCR-HDDD). Because of a retrospective study, patients were not required to write consent forms.

Consent to publish

Not applicable.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that no conflicts of interest exist.

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None.

Authors’ contributions

Vinh Thanh Tran and Thang Thanh Phan are senior authors who contributed to study design; Thang Thanh Phan, Tung Thanh Tran, and Tin Huu Vo selected patients for the study and collected clinical data; Hong-Phuoc Mac, Toan Trong Ho, Suong Phuoc Pho, Van-Anh Ngoc Nguyen, Truc-My Vo, Hue Thi Nguyen, and Thao Thi Le collected the laboratory data; Thang Thanh Phan, Vinh Thanh Tran, and Son Truong Nguyen performed the data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Table

Table 1. Diagnostic values of each marker for the APL

Antigen	Cut-off	AUC (95%CI)	Sensitivity, % (95%CI)	Specificity, % (95%CI)
CD117	≥49%	0.76 (64.0–88.0)	72.2 (56.3–87.5)	75.9 (59.1–90.9)
CD13	≥88%	0.84 (0.73–0.95)	90.6 (81.2–100.0)	68.2 (50.0–86.4)
CD56	≤25%	0.65 (0.54–0.76)	41.4 (23.5–61.1)	91.7 (77.5–98.3)
CD64	≥42%	0.82 (71.3–93.5)	84.4 (71.9–96.9)	72.7 (54.6–90.9)
MPO	≥97%	0.85 (0.75–0.94)	63.9 (50.0–81.3)	89.7 (77.3–100.0)

Figures

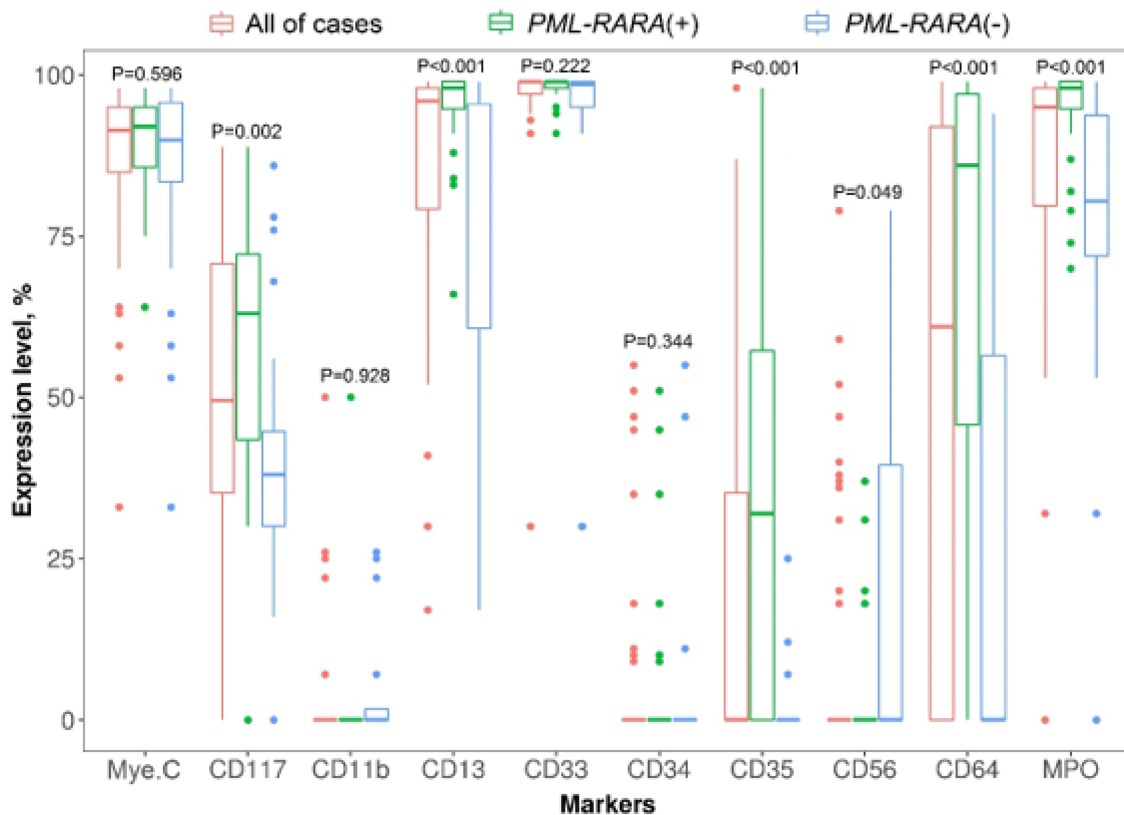

Figure 1

Antigen expression level between groups of PML-RARA status

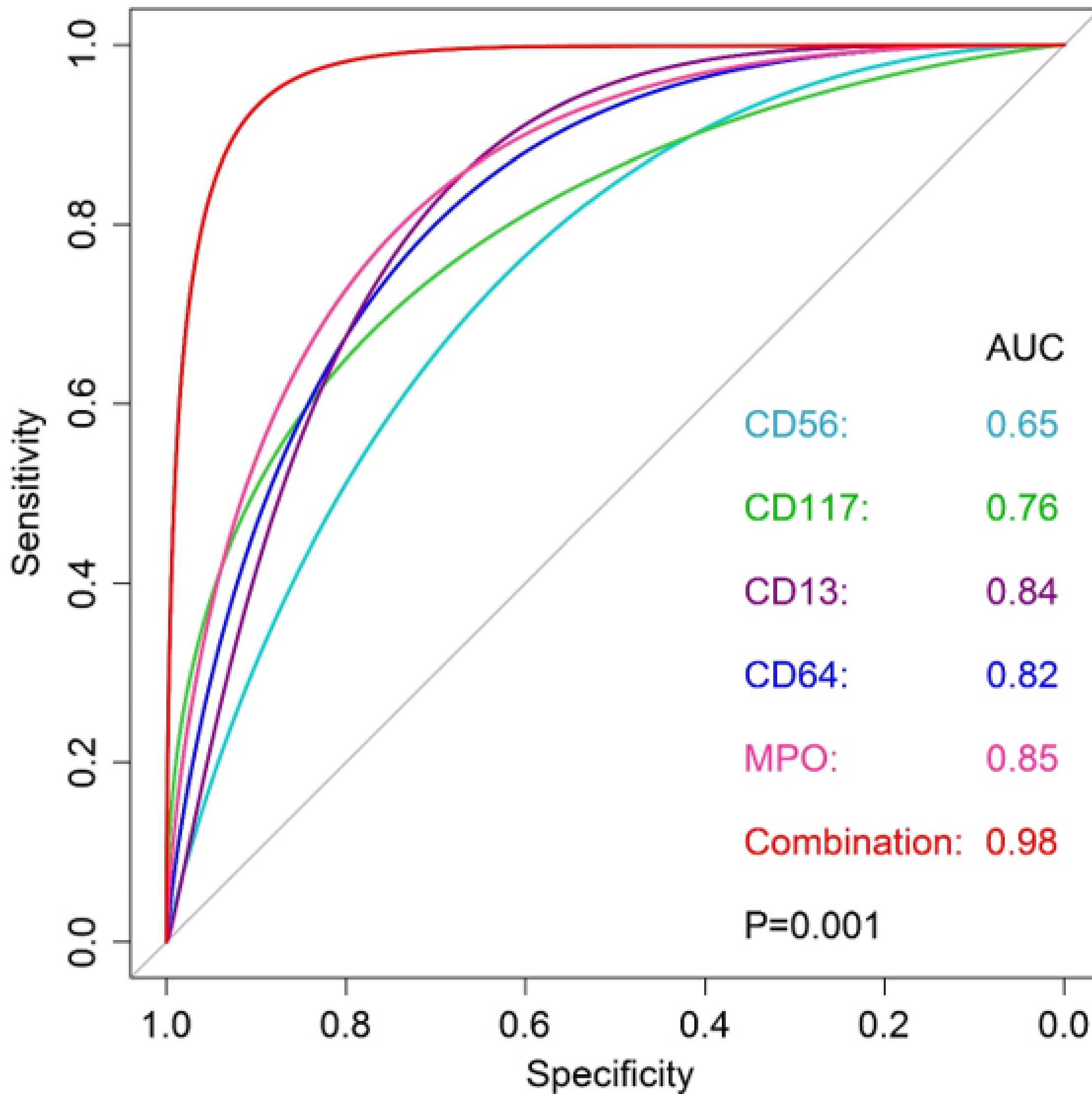

Figure 2

The diagnostic power of CD117, CD13, CD56, CD64, and MPO in combination

Supplementary Files

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