



CHIP variants and immunophenotypic features of HLA-DR(–) acute myeloid leukemia without *PML::RARA* fusion gene

Youngeun Lee¹ and Hye Ryou Kim^{2,*}

¹Department of Laboratory Medicine, Chungbuk National University Hospital, Cheongju, Korea

²Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Korea

Purpose: HLA-DR(–) is a well-known characteristic of acute promyelocytic leukemia (APL), but also observed in some acute myeloid leukemia (AML) patients without *PML::RARA* fusion gene. We investigated the prevalence of clonal hematopoiesis of indeterminate potential (CHIP), immunophenotypic and cytogenetic abnormalities of HLA-DR(–) AML patients by comparing with those of APL patients.

Materials and Methods: Thirteen patients diagnosed with HLA-DR(–) AML were enrolled. Twenty patients diagnosed with APL were assigned as a control group. Next-generation sequencing of CHIP genes (58 genes) was performed. Bone marrow or peripheral blood samples were used for flow cytometry to immunophenotype the leukemic blasts according to the laboratory protocol.

Results: In HLA-DR(–) AML patients, CD117 positivity was less frequent. CD13 showed lower expression level. The median number of variants was three per patient, which was significantly higher than in the APL group. *NPM1* variants were found in five patients. *FLT3-ITD* was frequently co-existed with *NPM1* variant.

Conclusion: HLA-DR(–) AML patients exhibit a higher frequency of complex genetic abnormalities (three or more), which may explain why their prognosis is worse than that of APL patients, despite the frequent observation of *NPM1*. It is necessary to gather the results for each gene and conduct further evaluation.

Key words: HLA-DR antigens, Clonal hematopoiesis, Immunophenotyping, Leukemia, promyelocytic, acute.

Introduction

HLA-DR expression appears in various types of leukemias and lymphoma. In particular, acute promyelocytic leukemia (APL) showed HLA-DR(–), a characteristic feature of APL from other non-APL acute myeloid leukemia (AML). Accurate and rapid diagnosis is essential as patients with APL could progress to an aggressive disease course through coagulopathy. APL has some

characteristic features identified by flow cytometry. Typically, abnormal promyelocytes showed low or absent expression of HLA-DR and CD34. Heterogeneous expression of CD13 and homogeneous bright expression of CD33 were also well known findings. However, HLA-DR expression loss is rarely observed in non-APL AML [1–3]. These HLA-DR(–) non-APL AML (HLA-DR[–] AML) patients showed distinct features such as high CXCR-4 expression, cup-shaped nucleus, loss of CD2 and CD34, increased

Received: 26 April 2025, Revised: 28 May 2025, Accepted: 14 June 2025, Published: 30 June 2025

*Corresponding author: Hye Ryou Kim, Ph.D. <https://orcid.org/0000-0002-9229-9665>

Department of Laboratory Medicine, Chung-Ang University College of Medicine, 102 Heukseok-ro, Dongjak-gu, Seoul 06973, Korea.

Tel: +82-2-6299-2718, Fax: +82-2-6299-2818, E-mail: hyekim@cau.ac.kr

Conflict of interest: The authors declare that they do not have any conflicts of interest.

© This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

© Copyright 2025 by the Korean Society of Medical Genetics and Genomics

www.e-kjgm.org

leukocyte and platelet count, and high frequency of *FLT3-ITD* and *NPM1* variants [1,2,4]. Clonal hematopoiesis of indeterminate potential (CHIP) occurs in aging and the most frequently mutated genes are *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2*, and so on. CHIP is thought to increase the risk of hematologic malignancy, cardiovascular disease, and stroke. CHIP is associated with diverse clinical outcomes, with clone size, specific gene, and inherent patient characteristics important mediators of risk [5]. However, few studies have been conducted on CHIP genetic variants of HLA-DR(–) AML patients. In this study, we aimed to identify characteristics of HLA-DR(–) AML patients, particularly on genetic variants. Also, we analyzed immunophenotypic and cytogenetic abnormalities to find the correlation.

Materials and Methods

1. Study population and clinical diagnosis

Thirteen patients diagnosed with HLA-DR(–) AML from May 2011 to May 2022 at Chung-Ang University Hospital were enrolled. Twenty patients diagnosed with APL during the same period were assigned as a control group. We used French-American-British (FAB) classification and World Health Organization (WHO) classification simultaneously and wrote both diagnosis together in the bone marrow (BM) reports. Initial diagnosis was mainly established by morphology, flow cytometric immunophenotyping and cytogenetic studies. We retrospectively reviewed medical records to obtain clinical information and laboratory test results such as complete blood count (CBC), BM reports, and cytogenetic studies. The study was approved by the Institutional Review Board of Chung-Ang University Hospital (2110-031-481). The requirement to obtain informed consent was waived.

2. Flow cytometry

BM or peripheral blood samples were used for flow cytometry to immunophenotype the leukemic blasts according to the laboratory protocol. The data acquisition and analysis were performed with Navios EX Flow Cytometer (Beckman Coulter). We used a panel of the following markers: CD13, CD33, CD117, MPO, CD34, CD3, CD7, CD5, CD2, CD19, CD20, cytoplasmic CD22 (cyCD22), cytoplasmic CD79a (cyCD79a), CD10, HLA-DR, TdT, CD14, CD61, and CD56 (Beckman Coulter). Of which, CD34 was added to the panel in 2015. The expression of antibodies were considered positive if most of these markers were expressed in more than 20% of gated population, except MPO, CD61, and TdT, which were considered positive when exceed 10%.

3. Deep targeted sequencing for CHIP

1) Targeted sequencing

Genomic DNA was purified from BMs using Genomic DNA Purification Kit (Promega). We customized a panel of 58 genes frequently mutated in hematologic malignancy. Genes included in the panel has been added to the end of the sentence. The primer was designed using Ion AmpliSeq™ Designer (Thermo Fisher Scientific) software. Libraries were prepared using Ion AmpliSeq™ Library Kit 2.0 (Thermo Fisher Scientific) on Ion Chef™ (Thermo Fisher Scientific) instrument according to the instructions. Ion Chef™ instrument automatically performed template preparation and chip loading using Ion 530™ Kit-Chef (Thermo Fisher Scientific) and Ion 530™ Chip Kit (Thermo Fisher Scientific) that were sequenced by Ion S5™ XL System (Thermo Fisher Scientific) sequencer.

2) Variant filtering

From the TSV files, the single-nucleotide variants and small insertions-deletions variants were retained according to the fol-

ABL1	CEBPA	GNB1	MPL	PTPN11	STAG1
ANKRD26	CSF3R	HRAS	MYD88	RAD21	STAG2
ASXL1	DDX41	IDH1	NF1	RB1	STAT3
ATRX	DNMT3A	IDH2	NOTCH1	RUNX1	TET2
BCOR	ETV6	IKZF1	NPM1	SETBP1	TP53
BCORL1	EZH2	JAK2	NRAS	SF3B1	U2AF1
BRAF	FLT3	JAK3	PDGFRA	SH2B3	WT1
CALR	GATA1	KDM6A	PHF6	SMC1A	ZRSR2
CBL	GATA2	KIT	PPM1D	SMC3	
CBLB	GNAS	KRAS	PRPF8	SRSF2	

lowing strategy: Phred quality score ≥20, altered read numbers ≥10, variant allele frequency (VAF) of 2% or higher [6], non-strand-biased variants (forward and reverse stranded alternative counts have a ratio between 3:7 and 7:3), coding region ±10 base pairs, and non-synonymous variants. The variants with minor allele frequency ≥1% in Genome Aggregation Database (gnomAD) v2.1 or Korean Reference Genome Database were removed. The variants that were included in the "Panel of Normals" using a sample from a healthy person were omitted. To get rid of false-positive variants, the single nucleotide deletion or insertion within homopolymer stretch and the trinucleotide deletion or insertion within the trinucleotide repeat-rich region which were repeatedly discovered with low VAF in our study cohort were excluded. Finally, the variants reported in ClinVar as "Benign" or "Likely benign" were filtered. Effects on splicing were predicted using SpliceAI. COSMIC (v94) was annotated in the

final variant list. The "Hotspot" variant was designated when it occurred in the COSMIC "haematopoietic and lymphoid" tissue category greater than five times.

4. Statistical analysis

Statistical analyses were performed using the R statistical program (<https://www.r-project.org>). The data are presented as the median (range) for continuous variables and the number of cases (percentages) for categorical variables. Statistical significance of continuous variables was calculated by Wilcoxon rank sum test and that of categorical variables was calculated by Fisher's exact test. Correlation coefficient and *P*-value between factors such as immunophenotype, cytogenetic, and mutated genes were calculated also by R program and false discovery rate correction. *P*-value <0.05 was considered statistically significant.

Results

1. Clinical characteristics of patients

The clinical features of patients are summarized in Table 1. In HLA-DR(-) AML patients, the median age at diagnosis is 66 years. The median value of CBC was a hemoglobin level of 8.4 g/dL (range, 6.6–11.5 g/dL), white blood cell count of $20.4 \times 10^9/L$ (range, 1.1 – $383.2 \times 10^9/L$), and platelet count of $69 \times 10^9/L$ (range, 10 – $135 \times 10^9/L$). The median percentage of BM blasts was 77.0% (range, 32.8–96.0%). Diagnosis based on the FAB classification was evenly distributed as M1, M2, M5, and M7. Considering cytogenetic and molecular abnormalities, nine patients were reclassified as AML with recurrent genetic abnormalities and three patients as AML with myelodysplasia-related changes. The median number of variants was three per patient. Among

Table 1. Clinical characteristics of HLA-DR(-) acute myeloid leukemia patients and acute promyelocytic leukemia patients

	HLA-DR(-) AML (n=13)	APL (n=20)	<i>P</i> -value
Age at diagnosis (yr)	66 (48-89)	54 (11-82)	0.0653
Sex (male/female)	9/4 (69.2)	11/9 (55.0)	0.4851
WBC ($\times 10^9/L$)	20.4 (1.1-383.2)	2.8 (0.4-142.8)	0.0868
Hemoglobin (g/dL)	8.4 (6.6-11.5)	8.2 (5.5-12.0)	0.5187
Platelets ($\times 10^9/L$)	69 (10-135)	52 (13-133)	0.9266
BM blasts	77.0 (32.8-96.0)	88.2 (60.0-95.2)	0.1784
FAB classification			<0.0001*
M0	1 (7.7)	0 (0.0)	
M1	3 (23.1)	0 (0.0)	
M2	3 (23.1)	0 (0.0)	
M3	0 (0.0)	20 (100.0)	
M5	3 (23.1)	0 (0.0)	
M7	3 (23.1)	0 (0.0)	
WHO classification			<0.0001*
AML, NOS	1 (7.7)	0 (0.0)	
AML with MRC	3 (23.1)	0 (0.0)	
APL with <i>PML::RARA</i>	0 (0.0)	20 (100.0)	
Variant <i>RARA</i> translocation	1 (7.7)	0 (0.0)	
AML with <i>KMT2A::MLLT3</i>	1 (7.7)	0 (0.0)	
AML with <i>KMT2A::MLLT1</i>	1 (7.7)	0 (0.0)	
AML with <i>NPM1</i> mutation	5 (38.5)	0 (0.0)	
AML with <i>RUNX1</i> mutation	1 (7.7)	0 (0.0)	
Variants	3 (0-8)	1 (0-4)	0.0046*
<i>NPM1</i>	5 (38.5)	0 (0.0)	0.0054*
<i>FLT3-ITD</i>	4 (30.8)	2 (10.0)	0.36
Deceased	9 (90.0)	6 (30.0)	0.0144 ^{a,*}
Median survival (mon)	2.5 (1-16)	39 (1-132)	0.0052 ^{a,*}

Values are presented as median (range) for continuous variables or number (%) for categorical variables.

^aPatients who could not follow up due to transfer were excluded.

**P*<0.05.

APL, acute promyelocytic leukemia; AML, acute myeloid leukemia; WBC, white blood cell; BM, bone marrow; FAB, French-American-British; WHO, World Health Organization; NOS, not otherwise specified; MRC, myelodysplasia-related changes.

the mutated genes, *NPM1* was the most commonly observed (38.5%). The median overall survival (OS) was 2.5 months.

In APL patients, the median age at diagnosis is 54 years. CBC values showed no significant difference from HLA-DR(–) AML patients. The median number of variants was one per patient, which was significantly smaller. The median OS was 39 months, significantly longer than the patient group.

2. Immunophenotypic features and differences between groups

We compared the expression profiles of immunophenotyping analysis between HLA-DR(–) AML and APL patients (Table 2). CD34, a marker of early haematopoietic stem cells, positivity was 10% in HLA-DR(–) AML patients. Expression of myeloid lineage-associated markers such as MPO, CD13, and CD33 was detected in most cases. However, CD117, an antigen expressed in hematopoietic progenitor cells, was detected less frequently in HLA-DR(–) AML patients (69.2%) than in APL patients (100.0%). CD14 and CD61 were markers of monocytic and megakaryocytic differentiation, respectively, and expression was detected only in HLA-DR(–) AML patients. Aberrant expression of T-cell markers such as CD2, CD3, CD5, and CD7 was all negative in HLA-DR(–) AML patients and observed only in APL patients. Only CD2 showed a difference between the two groups, CD3, CD5 and CD7 were negative in both. Aberrant expression of other lymphoid markers was rarely observed. CD56 was positive in 23% of HLA-DR(–) AML patients and 10% of APL patients.

When comparing expression intensity (%), CD13 and CD19 expression levels were lower in HLA-DR(–) AML patients than in APL patients. The expression intensity of CD56 and CD61 was higher in the HLA-DR(–) AML patients.

3. CHIP gene variants in HLA-DR(–) AML patients and correlation between cytogenetics and variants

In HLA-DR(–) AML patients, 46 variants were observed in 23 genes (Fig. 1). The number of variants was a median of 3 per patient (range, 0–8). *NPM1* and *FLT3* variants were the most common. All *NPM1* variants were frameshift variants in tryptophans (W) 288. Four out of 5 patients with *NPM1* variants also had *FLT3-ITD*. After *NPM1* and *FLT3*, *ANKRD26*, *NRAS*, and *IDH2* variants were frequently detected.

In APL patients, 26 variants were observed in 15 genes. The number of variants was a median of 1 per patient (range, 0–4). *FLT3* variants, including *FLT3-ITD*, were detected in 7 out of 20 patients, the most frequently mutated gene. Other than *FLT3*, variants in genes such as *ANKRD26*, *PPM1D*, *PDGFRA*, and *WT1*

Table 2. Comparison of immunophenotype positivity and antigen expression levels in the gated leukemic cells between groups

	HLA-DR(–) AML (n=13)	APL (n=20)	P-value
HLA-DR			
Positive	0/13 (0.0)	0/20 (0.0)	NA
Median (range)	6.3 (0.0–19.2)	0.7 (0.1–17.3)	0.0034#
Myeloid lineage			
CD34 ^a			
Positive	1/10 (10.0)	3/11 (27.3)	0.5865
Median (range)	2.1 (0.3–99.6)	4.9 (0.2–94.9)	0.8326
CD13			
Positive	12/13 (92.3)	20/20 (100.0)	0.3939#
Median (range)	86.3 (13.2–96.0)	98.1 (66.5–99.9)	0.0024#
CD33			
Positive	13/13 (100.0)	20/20 (100.0)	NA
Median (range)	95.9 (88.1–100.0)	99.5 (66.3–100.0)	0.1548
CD117			
Positive	9/13 (69.2)	20/20 (100.0)	0.0175*
Median (range)	78.2 (0.1–96.0)	77.8 (32.4–97.1)	0.4555*
MPO			
Positive	10/13 (76.9)	20/20 (100.0)	0.0524
Median (range)	92.9 (2.0–99.7)	96.5 (61.6–99.9)	0.8654
T-cell lineage			
CD2			
Positive	0/13 (0.0)	4/20 (20.0)	0.1359
Median (range)	0.2 (0.0–7.0)	0.6 (0.0–89.7)	0.0586
B-cell lineage			
CD19			
Positive	0/13 (0.0)	3/20 (15.0)	0.2614#
Median (range)	0.2 (0.0–4.3)	1.4 (0.0–58.6)	0.0421#
cCD79a			
Positive	1/13 (7.7)	1/20 (5.0)	1
Median (range)	1.2 (0.1–51.4)	0.9 (0.0–36.7)	0.4949
Others			
TdT			
Positive	0/13 (0.0)	1/20 (5.0)	1
Median (range)	1.0 (0.0–6.8)	0.1 (0.0–11.2)	0.0779
CD14			
Positive	3/13 (23.1)	0/20 (0.0)	0.0524
Median (range)	0.5 (0.0–82.4)	0.7 (0.1–11.5)	0.7394
CD56			
Positive	3/13 (23.1)	2/20 (10.0)	0.36#
Median (range)	2.6 (0.4–90.5)	0.5 (0.0–78.3)	0.0191#
CD61			
Positive	5/13 (38.5)	0/20 (0.0)	0.0054*#
Median (range)	7.4 (0.9–44.0)	1.9 (0.4–9.6)	0.0017*#

Values are presented as median (range) or number (%).

^aWhen calculating the P-value of CD34 results, we excluded the patients diagnosed with the disease before 2015 because CD34 results were unavailable until 2015.

*P<0.05, comparison of immunophenotype positivity between groups; #P<0.05, comparison of expression levels of antigens between groups.

APL, acute promyelocytic leukemia; AML, acute myeloid leukemia; NA, not applicable.

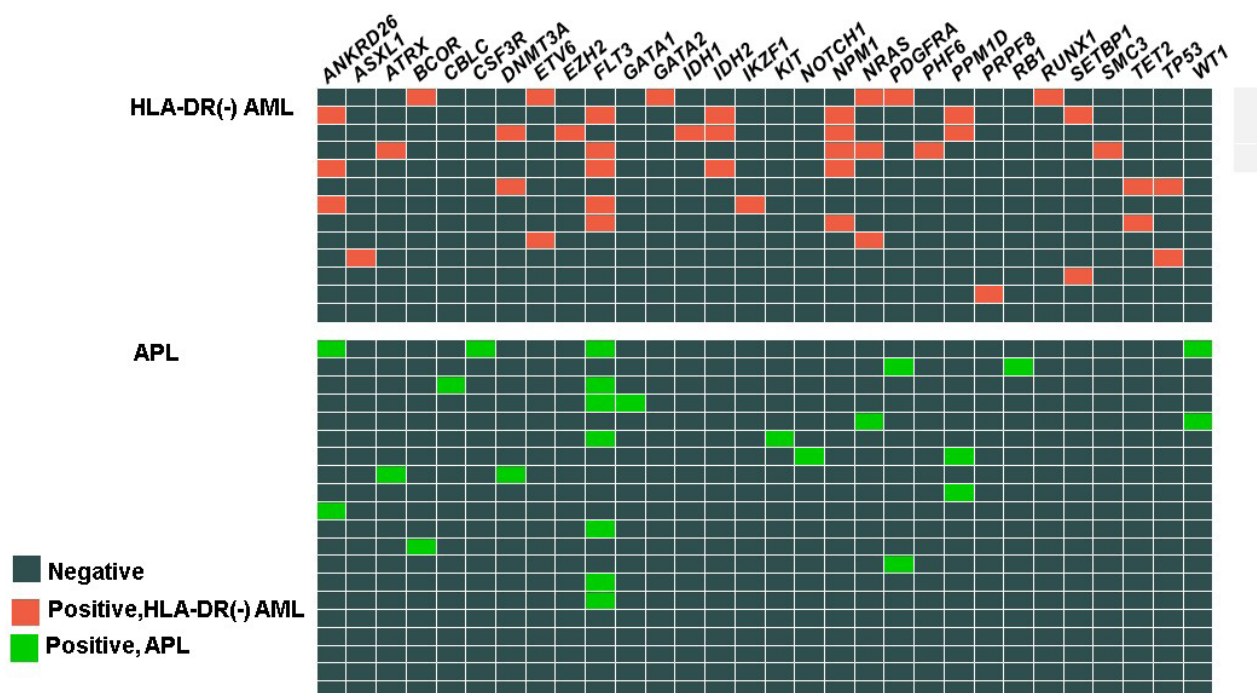


Fig. 1. Distribution of mutated genes in HLA-DR(-) AML patients (upper) and APL patients (lower). AML, acute myeloid leukemia; APL, acute promyelocytic leukemia.

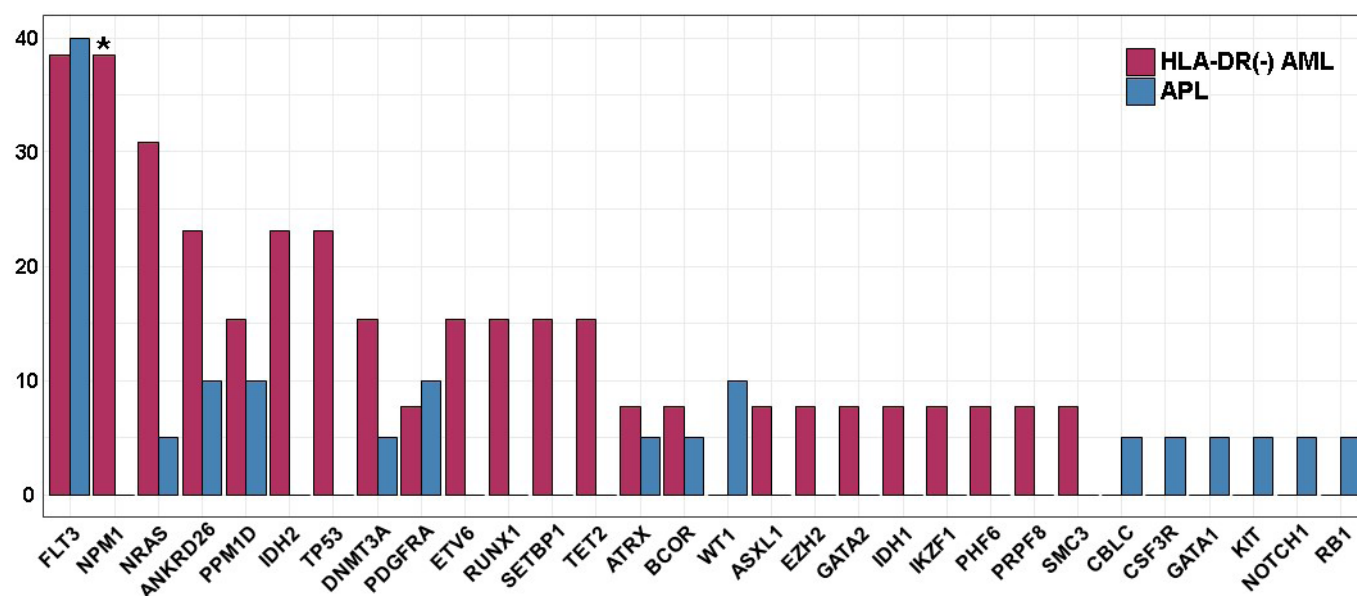


Fig. 2. Comparison of frequency of mutated genes between groups. *NPM1* variant is significantly increased in HLA-DR(-) AML patients than APL patients with *PML::RARA* fusion. * $P < 0.05$. AML, acute myeloid leukemia; APL, acute promyelocytic leukemia.

were relatively more frequent (2/20) than others.

When comparing the frequency of mutated genes between the two groups, the frequency of *NPM1* variants was significantly higher in HLA-DR(-) AML patients than in APL patients (Fig. 2). We subdivided the patient group based on the presence of *NPM1* variants. *FLT3* and *IDH2* genes, including *FLT3-ITD* vari-

ant, were more frequent in patients with *NPM1* variants than patients without *NPM1* variants.

Discussion

Diagnosis of AML has been based on a comprehensive analysis

of morphology, immunophenotype, cytogenetic, and molecular tests. The recently revised 5th WHO classification included more diverse fusion genes as disease-defining genetic abnormalities. They emphasized the importance of detecting recurrent genetic abnormalities rather than traditional blast counts by morphology [7]. However, not all clinical laboratories can perform high-throughput molecular testing, and those cytogenetic and molecular testing could take weeks to get final reports. As AML patients need quick identification of the disease at an early stage and choose the appropriate treatment regimen, flow cytometric immunophenotyping has been widely used to identify the lineage of leukemic blasts and aberrant expression. Immunophenotyping also provides some clues about genetic abnormalities. For example, low or absent expression of HLA-DR and CD34, homogeneous bright expression of CD33, and heterogeneous expression of CD13 are notable features in APL [8]. However, these immunophenotypic features alone cannot clearly distinguish APL and other AML types [1,9]. Approximately 5–20% of newly diagnosed patients with AML showed HLA-DR negativity without *PML::RARA* fusion [1–4,10–12]. Previous studies reported several distinctive features of these HLA-DR(–) AML patients. Oelschlaegel et al. [2] reported that high CXCR-4 expression is the main differential biological characteristic of HLA-DR(–) AML. The exclusive presence of *NPM1* variants, cup-like morphology, and higher leukocyte counts also showed biological distinctness between HLA-DR(–) AML and APL groups. There have been several studies that HLA-DR(–) AML patients show characteristics similar to the variant APL [1,2] or intermediate features between those of patients with typical AML and APL [3]. The high frequency of *NPM1* variant and co-occurrence of *FLT3-ITD* were also a hallmark of the disease [3,10,13]. *NPM1*-mutated AML patients are usually associated with a normal karyotype and mutually exclusive of other recurrent genetic abnormalities [8]. Blasts cells usually show CD34 and HLA-DR negativity. Secondary variants are common in *FLT3* and *DNMT3A*, following *IDH1*, *KRAS*, and *NRAS* [13,14]. In this study, five patients with *NPM1* variant were identified. Four out of five patients also had *FLT3-ITD*, and those patients showed both HLA-DR and CD34 negativity. Other than *FLT3-ITD*, additional variant was found in *IDH2*, *ANKRD26*, and *PPM1D*. HLA-DR(–) AML patients without *NPM1* variant mostly showed other recurrent cytogenetic abnormalities or complex karyotype by G-banding analysis and FISH studies. These patients showed a heterogeneous distribution of a relatively lower number of variants. The most common variant was identified in two out of eight patients in the mutated genes, such as *NRAS*, *ETV6*, and *TP53*. The

number of variants in the *NPM1*-mutated cases tended to be slightly higher, but it was not statistically significant ($P=0.052$). A notable finding of this study is that HLA-DR(–) AML patients exhibit a higher frequency of complex genetic abnormalities (three or more), which may explain why their prognosis is worse than that of APL patients, despite the frequent observation of *NPM1*, a favorable prognostic factor. It is considered even more meaningful as a study utilizing the CHIP gene, a key risk factor for hematologic malignancies. However, various cytogenetic translocations could also be detected in these patients. If we concluded the diagnosis based solely on the immunophenotype and cytogenetics without further molecular analysis, patients with higher variant burden could be missed.

We compared the two patient groups based on their expression of flow cytometric markers. In leukemic blasts, CD117 expression positivity was lower than that of APL patients. CD13 expression was mainly positive in both patient groups. However, the range was relatively lower in HLA-DR(–) AML patients. These features, that is, HLA-DR negativity and weak and heterogeneous expression of CD13 compared to CD33, have been observed in HLA-DR(–) AML patients [3], and are also well-known diagnostic features of APL. Because there are no characteristic markers, it is challenging to distinguish APL from other AML types according to immunophenotype. Wetzler et al. [1] reported that diagnosing APL required cytogenetic or molecular confirmation in addition to morphology and HLA-DR antigen expression. CD2 negative expression was reported as a characteristic of HLA-DR(–) AML compared to APL in previous studies [2,3]. In this study, CD2 aberrant expression was only observed in APL patients, but there was no statistically significant difference. It might be helpful to use several markers simultaneously to obtain diagnostic clues until the results of fusion gene tests are reported [15].

In conclusion, HLA-DR negative (–) AML patients exhibit immunophenotypic features highly similar to those of APL patients, but show differences in CHIP variants. The most commonly mutated gene was *NPM1*, and *FLT3-ITD* frequently co-existed in these patients. A notable finding of this study is that HLA-DR(–) AML patients exhibit a higher frequency of complex genetic abnormalities (three or more), which may explain why their prognosis is worse than that of APL patients, despite the frequent observation of *NPM1*, a favorable prognostic factor. It is necessary to gather the results for each gene and conduct further evaluation.

Acknowledgements

None.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C2013359).

Authors' Contributions

Conception and design: HRK. Acquisition of data: YL. Analysis and interpretation of data: YL. Drafting the article: YL. Critical revision of the article: HRK. Final approval of the version to be published: HRK.

References

1. Wetzler M, McElwain BK, Stewart CC, Blumenson L, Mortazavi A, Ford LA, et al. HLA-DR antigen-negative acute myeloid leukemia. *Leukemia* 2003;17:707–15.
2. Oelschlaegel U, Mohr B, Schaich M, Schäkel U, Kroschinsky F, Illmer T, et al. HLA-DRneg patients without acute promyelocytic leukemia show distinct immunophenotypic, genetic, molecular, and cytomorphic characteristics compared to acute promyelocytic leukemia. *Cytometry B Clin Cytom* 2009;76:321–7.
3. Lee HY, Cho YU, You E, Jang S, Seo EJ, Park CJ. Frequency and distinct characteristics of acute myeloid leukemia lacking HLA-DR and CD34 expression: features intermediate between typical acute myeloid leukemia and acute promyelocytic leukemia. *Lab Med Online* 2017;7:103–10.
4. Moon H, Lee S, Huh J, Chung WS. Characteristics of acute myeloid leukemia without HLA-DR expression. *Korean J Lab Med* 2007;27:313–7.
5. Singh J, Li N, Ashrafi E, Thao LTP, Curtis DJ, Wood EM, et al. Clonal hematopoiesis of indeterminate potential as a prognostic factor: a systematic review and meta-analysis. *Blood Adv* 2024;8:3771–84.
6. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hassarjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* 2015;126:9–16.
7. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia* 2022;36:1703–19.
8. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer Publications; 2017.
9. Gupta A, Mishra D, Chandy M, Parihar M. Human leucocytic antigen-DR negative acute myeloid leukemia: a diagnostic dilemma for hematopathologist. *Clin Cancer Investig J* 2014;3:383–7.
10. Gajendra S, Gupta R, Thakral D, Gupta SK, Jain G, Bakhshi S, et al. CD34 negative HLA-DR negative acute myeloid leukaemia: a higher association with NPM1 and FLT3-ITD mutations. *Int J Lab Hematol* 2023;45:221–8.
11. Kaleem Z, Crawford E, Pathan MH, Jasper L, Covinsky MA, Johnson LR, et al. Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. *Arch Pathol Lab Med* 2003;127:42–8.
12. Zheng J, Wang X, Hu Y, Yang J, Liu J, He Y, et al. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. *Cytometry B Clin Cytom* 2008;74:25–9.
13. Wang B, Yang B, Wu W, Liu X, Li H. The correlation of next-generation sequencing-based genotypic profiles with clinicopathologic characteristics in NPM1-mutated acute myeloid leukemia. *BMC Cancer* 2021;21:788.
14. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med* 2005;352:254–66.
15. Tran VT, Phan TT, Mac HP, Tran TT, Ho TT, Pho SP, et al. The diagnostic power of CD117, CD13, CD56, CD64, and MPO in rapid screening acute promyelocytic leukemia. *BMC Res Notes* 2020;13:394.