Brief Communication
Diagnostic Hematology

Ann Lab Med 2023;43:503-507
https://doi.org/10.3343/alm.2023.43.5.503
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Implications of the 5th Edition of the World Health Organization Classification and International Consensus Classification of Myeloid Neoplasm in Myelodysplastic Syndrome With Excess Blasts and Acute Myeloid Leukemia

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The fifth edition of the WHO classification (2022 WHO) and the International Consensus Classification (2022 ICC) of myeloid neoplasms have been recently published. We reviewed the changes in the diagnosis distribution in patients with MDS with excess blasts (MDS-EB) or AML using both classifications. Forty-seven patients previously diagnosed as having AML or MDS-EB with available mutation analysis data, including targeted next-generation and RNA-sequencing data, were included. We reclassified 15 (31.9%) and 27 (57.4%) patients based on the 2022 WHO and 2022 ICC, respectively. One patient was reclassified as having a translocation categorized as a rare recurring translocation in both classifications. Reclassification was mostly due to the addition of mutation-based diagnostic criteria (i.e., AML, myelodysplasia-related) or a new entity associated with *TP53* mutation. In both classifications, MDS diagnosis required the confirmation of multi-hit *TP53* alterations. Among 14 patients with *TP53* mutations, 11 harbored multi-hit *TP53* alterations, including four with *TP53* mutations and loss of heterozygosity. Adverse prognosis was associated with multi-hit *TP53* alterations ($P=0.009$) in patients with MDS-EB, emphasizing the importance of detecting the mutations at diagnosis. The implementation of these classifications may lead to the identification of different subtypes from previously heterogeneous diagnostic categories based on genetic characteristics.

Key Words: Myelodysplastic syndrome, Acute myeloid leukemia, Prognosis, World Health Organization, Mutation, *TP53*

Received: December 6, 2022
Revision received: January 16, 2023
Accepted: March 23, 2023

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Genome studies of myeloid hematological malignancies, including AML and MDS, have advanced our understanding of these diseases and revealed molecularly distinct groups [1–4]. Recently, the fifth edition of the WHO classification (2022 WHO) and the International Consensus Classification (2022 ICC) of myeloid neoplasms were published [5, 6]. In both classifications, the major changes regarding AML and MDS include: (1) changes in the blast thresholds defining AML, including the expansion of the genetic abnormality categories that may be diagnosed as AML with a blast cut-off requirement of 10%; (2) the introduction of an AML classification subtype termed "rare recurring translocation"; and (3) the extension of mutation-based defini-

tions [5–7]. We reclassified patients diagnosed as having AML and MDS with excess blasts (MDS-EB) according to the 2016 WHO classification (2016 WHO), using the 2022 WHO and 2022 ICC. As the blast percentage defining MDS-EB and AML has been lowered and various genetic alterations have been introduced in both classifications, we reviewed the changes in the diagnosis distribution of patients with MDS-EB and AML using both the 2022 WHO and 2022 ICC. In addition, we focused on a newly introduced classification category, including *TP53* mutation.

Forty-seven adult patients diagnosed as having AML or MDS-EB between November 2019 and September 2021 with available mutation analysis data, including targeted next-generation and RNA-sequencing data, were included in our study. Patients with AML with 2016 WHO-designated recurring balanced translocations were excluded (Fig. 1). This study was approved by the Institutional Review Board of Korea University, Guro Hospital, Seoul, Korea (2021GR0247, 2021GR0572). Overall patient characteristics are provided in Supplemental Data Table S1.

We retrospectively reviewed the clinical responses of all patients and conducted a survival analysis. Cytogenetic abnormalities were analyzed according to the 2020 International System for Human Cytogenomic Nomenclature guidelines [8]. Sequence data were mapped to the reference genome GrCH37 (hg19). Mutation analysis was performed using the Oncomine Myeloid Research Assay (Thermo Fisher Scientific, Waltham, MA, USA). Libraries were prepared using the Ion Chef System (Thermo Fisher Scientific) and sequenced using an Ion S5 XL sequencer (Thermo Fisher Scientific). Alignment and base calling were performed using Torrent Suite (v5.10), and variant calling was performed using Ion Reporter (v5.10). The variant allele fraction (VAF) cut-off for missense and nonsense variants was 5% and 10%, respectively, for insertions/deletions. RNA-sequencing libraries were prepared using the TruSeq Stranded Total RNA LT sample preparation kit (Illumina, San Diego, CA, USA) and sequenced using the NovaSeq platform (Illumina). Sequencing reads were aligned using the STAR aligner (v2.6.0), and fusion transcripts were detected using Arriba (v2.2.0) [9, 10]. For samples with *TP53* mutations, a single-nucleotide polymorphism microarray analysis was performed using the CytoScan 750 K Assay (Affymetrix/Thermo Fisher). All statistical analyses were performed using R (v3.4.3; R Core Team, 2021) [11]. Detailed statistical methods are provided in the Supplemental Material.

Although the genetic abnormality categories that may be diagnosed as AML with a blast cut-off requirement of 10% have been expanded, patients with MDS-EB that were reclassified as AML were not observed. Among the 37 patients with AML, AML with recurrent genetic abnormalities accounted for 45.9% (17/37)

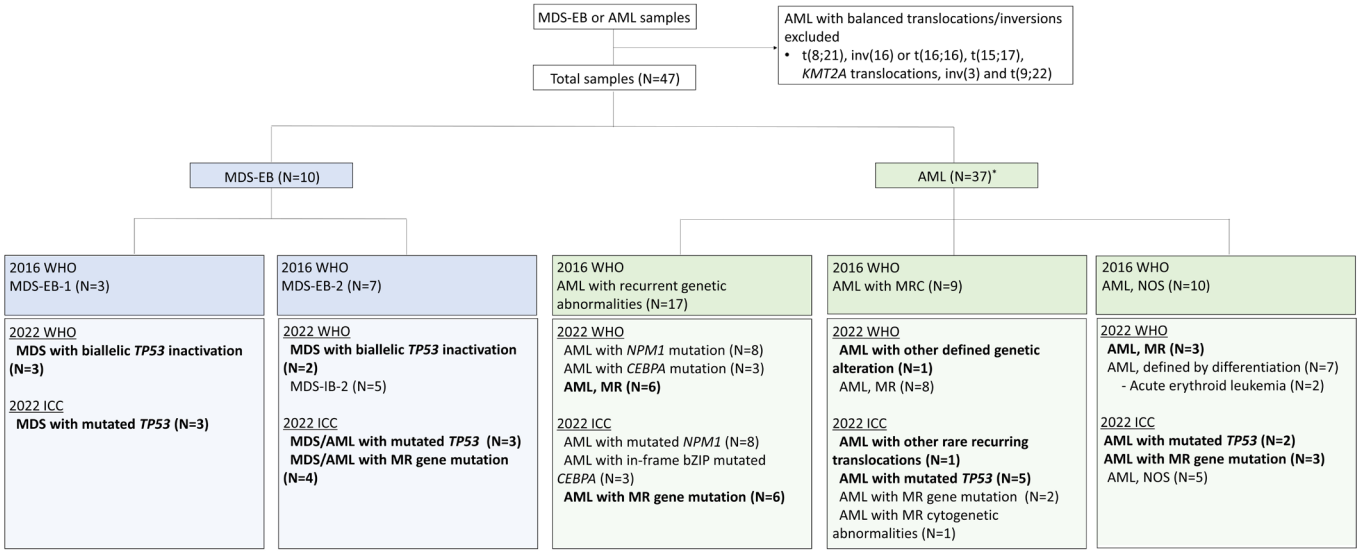

Fig. 1. Summary of the reclassification of the study population according to the 2022 WHO and 2022 ICC classifications. *A case diagnosed as therapy-related myeloid neoplasm according to the 2016 WHO classification is not included in the above figure. It was reclassified as AML, MR post cytotoxic therapy and AML with mutated *TP53*, therapy-related according to the 2022 WHO and 2022 ICC classifications, respectively.
Abbreviations: MDS-EB, MDS with excess blasts; ICC, International Consensus Classification; IB, increased blasts; MR, myelodysplasia-related; MRC, myelodysplasia-related changes; NOS, not otherwise specified.

https://doi.org/10.3343/alm.2023.43.5.503

according to the 2016 WHO, whereas 32.4% (12/37) of patients with AML were classified as AML with defining genetic abnormalities according to the 2022 WHO and as AML with recurrent genetic abnormalities according to the 2022 ICC (Fig. 2). Because of the exclusion of the provisional entity of AML with mutated *RUNX1* from AML with recurrent genetic abnormalities in the 2022 WHO and 2022 ICC, the diagnoses of six patients with AML with *RUNX1* mutations were modified. Notably, all of these six patients harbored mutations in myelodysplasia-related (MR) genes (e.g., *SRSF2, BCOR, ASXL1, SF3B1,* and *U2AF1*), and the diagnoses were reclassified to AML, MR according to the 2022 WHO, based on the mutation-based definition. In the 2022 ICC, *RUNX1* is considered an MR gene; therefore, the diagnosis was reclassified as AML with MR gene mutations. One patient was reclassified as AML with *FUS::ERG*, as the translocation was categorized as a rare recurring translocation in both classifications. RNA-sequencing did not reveal other rare recurring translocations included in the 2022 WHO or 2022 ICC, probably because translocations are rare, and numerous translocations that occur predominantly in infants and children were included as rare recurring translocations [6, 8].

AML with myelodysplasia-related changes (AML-MRC) accounted for 24.3% (9/37) of the patients with AML according to the 2016 WHO; an increase in patients classified as AML, MR according to the 2022 WHO (45.9%, N=17/37) and AML with MR gene mutation or AML with MR cytogenetic abnormalities according to the 2022 ICC (32.4%, N=12/37) was noted when compared to the classification according to the 2016 WHO (Fig. 2). With the introduction of the mutation-based definition of AML, MR, in addition to patients with AML with mutated *RUNX1*, three patients with AML, not otherwise specified were reclassified as MR AML according to both the 2022 WHO and the 2022 ICC.

TP53 alterations, especially *TP53* mutations, in MDS and AML are often associated with a complex karyotype and have highly adverse prognostic implications [3, 13–15]; therefore, the presence of *TP53* mutations is recognized as a new category in both the 2022 WHO and the 2022 ICC. In the 2022 ICC, a disease category of myeloid neoplasms with mutated *TP53* encompassing MDS, MDS/AML, and AML with mutated *TP53* has been newly introduced as a distinct category. Regarding the 2022 WHO, patients with MDS with multi-hit *TP53* alterations are classified as MDS with biallelic *TP53* (MDS-bi*TP53*), regardless of blast percentage; however, a distinct category for AML with mutated *TP53* was not defined. Based on the 2022 ICC, five patients with AML-MRC, two patients with pure erythroid leukemia, and one patient with therapy-related myeloid neoplasms were reclassified as patients with AML with *TP53* mutation, which accounted for 21.6% (8/37) of the patients with AML. Of the 10 patients with MDS-EB, five had multi-hit *TP53* alterations and one had a single *TP53* mutation. As multi-hit *TP53* alteration status is a diagnostic criterion for MDS-bi*TP53*, one patient with MDS-EB-2 with a single *TP53* mutation was classified as MDS-IB-2 according to the 2022 WHO, whereas the classification according to the 2022 ICC was MDS/AML with mutated *TP53*.

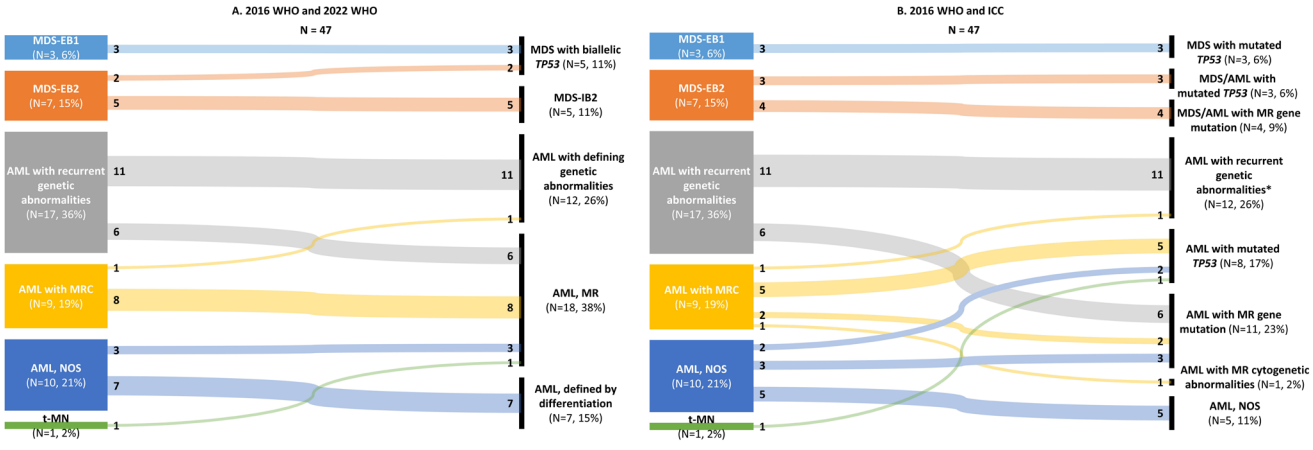

Fig. 2. Relationships between subtypes in the study population classified according to the 2016 WHO, 2022 WHO, and 2022 ICC classifications. Reclassification based on the (A) 2022 WHO and (B) 2022 ICC classifications. *AML with recurrent genetic abnormalities is not a valid category in the 2022 ICC classification. The term was adopted from the WHO to characterize eight AML cases with mutated *NPM1*, three AML cases with in-frame bZIP-mutated *CEBPA*, and one AML case with other rare recurring translocations.
Abbreviations: MDS-EB, MDS with excess blasts; MRC, myelodysplasia-related changes; NOS, not otherwise specified; t-MN, therapy-related myeloid neoplasms; IB, increased blasts; MR, myelodysplasia-related; ICC, International Consensus Classification.

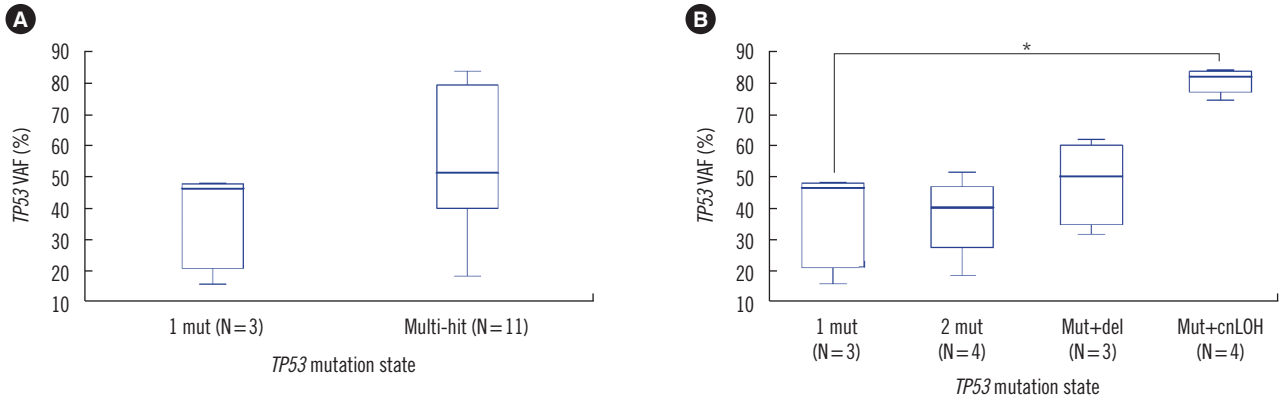

Fig. 3. Boxplots showing *TP53* VAF levels stratified by *TP53* mutation states. (A) Comparison of VAF levels between the single *TP53* mutation (1mut) and multi-hit *TP53* alteration groups. (B) Comparison of VAF levels between single *TP53* mutation and various subtypes of multi-hit *TP53* alterations. Multi-hit *TP53* alterations comprise two *TP53* mutations (2mut), a single *TP53* mutation with copy number loss of *TP53* (Mut+del), and a single *TP53* mutation with cnLOH (Mut+cnLOH). *$P<0.1$, Wilcoxon–Mann–Whitney test; each multi-hit *TP53* alteration subgroup was compared to the single *TP53* mutation group.
Abbreviations: VAF, variant allele fraction; cnLOH, copy number neutral loss of heterozygosity.

Among the 47 patients, 14 patients (six with MDS-EB, seven with AML, and one with therapy-related myeloid neoplasms) harbored *TP53* mutations. All of these 14 patients had a complex karyotype (≥3 chromosomal abnormalities), and 11 patients harbored multi-hit *TP53* alterations (three with two *TP53* mutations, four with one *TP53* mutation and copy number loss of *TP53*, and four with TP53 mutation and copy number neutral loss of heterozygosity [cnLOH]). *TP53* VAF, estimated by the maximum *TP53* VAF value for the multi-hit *TP53* alteration group, did not significantly differ between the single *TP53* mutation and multi-hit *TP53* alteration groups; however, patients with multi-hit *TP53* with cnLOH tended to have higher VAFs than those with a single *TP53* mutation ($P=0.057$; median VAF, 46.5% vs. 81.7%) (Fig. 3). We investigated the prognostic impact of *TP53* mutations on MDS-EB and AML using survival analysis. In 10 patients with MDS-EB, shorter overall survival (OS) was associated with *TP53* mutations ($P=0.006$; median OS 21.8 vs. 6.9 months) and multi-hit *TP53* alterations ($P=0.009$; median OS, 21.8 vs. 6.9 months) (Supplemental Data Fig. S1), but not with the EB-1 or EB-2 subtype ($P=0.159$). Univariate analyses showed that multi-hit *TP53* alterations, but not single *TP53* mutation, had a significantly greater hazard ratio (HR) of death (HR=10.77, $P=0.033$). Survival analysis of the 37 AML patients revealed that *TP53* mutations also had an adverse prognostic impact in AML patients, albeit with borderline significance ($P=0.072$; median OS 12.0 vs. 4.4 months) (Supplemental Data Fig. S1).

Using the 2022 WHO and 2022 ICC, we reclassified 15 (31.9%) and 27 (57.4%) out of 47 patients, respectively. This result was most likely a consequence of the addition of mutation-based definitions in the diagnostic criteria of AML, MR and the introduction of new entities, such as myeloid neoplasms with mutated *TP53*. The differences in the numbers of reclassified cases between the 2022 WHO and 2022 ICC categories were due to the inclusion of MR mutations in the previous category of MDS-EB2 and different entities of myeloid neoplasms with *TP53* mutation. These new classifications improved our diagnostic capability of defining a highly adverse prognostic group, especially in patients with MDS with multi-hit *TP53* alterations. With the increasing importance of detecting multi-hit *TP53* alterations in the diagnostic approach in both the 2022 WHO and 2022 ICC, the adaptation of various molecular test methods may be necessary in clinical settings, especially in cases of multi-hit alterations with low VAFs [13]. In addition, although with borderline significance, we observed an adverse prognostic impact of *TP53* mutations in patients with AML, suggesting that AML with mutated *TP53* may be a distinct group with poor prognosis, consistent with the 2022 ICC [6].

In summary, the 2022 WHO and 2022 ICC identified different subtypes from previously heterogeneous diagnostic categories based on genetic characteristics. Changes in the blast thresholds for defining AML in some genetic abnormality categories were not associated with the major reclassified cases in the clinical setting.

ACKNOWLEDGEMENTS

None.

https://doi.org/10.3343/alm.2023.43.5.503

AUTHOR CONTRIBUTIONS

Yoon SY and Yoon J contributed to the study conception and design; Kim HN, Kwon JA, Jeon MJ, Yu ES, Kim DS, and Choi CW were involved in clinical evaluation; Lee C, Yoon SY, and Yoon J interpreted the results; Lee C and Yoon J statistically analyzed the data; Lee C and Yoon J drafted the manuscript; and Yoon J supervised the study. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT) (2022R1G1A1007629) and a grant funded by Korea University (K2023141).

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