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Abstract:

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TO THE EDITOR:

Acute Myeloid Leukemia with Myelodysplasia Related Changes (AML-MRC) is a high-risk AML subtype with a reported frequency of 24-35% of all AMLs.[1-3] AMLs that develop after prior therapy (therapy-related myeloid neoplasms, t-AML), with recurrent genetic abnormalities and those with *NPM1* or biallelic *CEBPA* mutations, are excluded regardless of morphologic dysplasia.[4,5] AML-MRC diagnosis is straightforward when there is an antecedent history of Myelodysplastic syndrome (MDS) or myelodysplastic/Myeloproliferative neoplasm (MDS/MPN) or when dysplasia (> 50%) is present in at least 2 cell lineages. However, when these conditions are not met, the diagnosis depends on WHO defined MDS-associated cytogenetic abnormalities. In general, the complexity of modern hematopathology diagnoses requires the incorporation of cytogenetics/ fluorescence in-situ hybridization (FISH) and/or molecular findings in a timely fashion.[6]

AML-MRC, like t-AML, is associated with a poor prognosis [2] and lower response rates using conventional chemotherapy.[7-9] Based on better survival data, the FDA approved CPX-351 (Vyxeos; Jazz Pharmaceuticals), a fixed-dose liposomal formulation of daunorubicin and cytarabine for the treatment of AML-MRC and t-MRC.[7] Also, alternative treatments including lower intensity therapies (hypomethylating agents with or without venetoclax, or low-dose cytarabine plus either glasdegib or venetoclax) might be better options in older patients.[2] Early diagnosis of AML-MRC is crucial to make use of these newer therapies. While the history of MDS or MDS/MPN is usually available upfront, enough maturing non-blast hematopoietic cells for assessment of dysplasia might not be available. Regardless, for the latter, AML-MRC designation is contingent on excluding a *CEBPA* or *NPM1* mutation. Also, metaphase analysis and FISH results are generally not available at the time of initial diagnosis of AML; the turnaround time (TAT) of conventional chromosomal analysis ranges from 7-21 days.[10] Our aim was to identify the percentage of cases that qualified for a

diagnosis of AML-MRC solely based on MDS-associated cytogenetic abnormalities and thus, would have benefited from upfront CPX-351 induction chemotherapy or other alternative therapies.

We identified 64 AML-MRC cases with archived bone marrow samples (HFHS) over a period of 15 years. A previous history of MDS or MDS/MPN was present in 8% (5/64) of patients (Figure 1A). Only 30% patients (19/64) had more than 50% dysplasia in two or more lineages (Figures 1A, C-E). Remaining had either no dysplasia, dysplasia less than 50% and/or dysplasia in only one lineage or lacked sufficient differentiated cells to assess dysplasia. The most frequently reported dysplastic cell line was granulocytic (45%), followed by megakaryocytic (38%) and erythroid (16%). A striking 62% (40/64) of cases required MDS-associated cytogenetic abnormalities for AML-MRC diagnosis (Figure 1A). Of AML-MRC cases, 83% (53/64) had complex cytogenetics as defined by ≥3 unrelated abnormalities, 8% (5/64) had del(5q), 6% (4/64) had -7 or del(7q) and 3% (2/64) had other MDS associated abnormalities (Figure 1B). The study was approved by the Henry Ford Hospital Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Following these findings, a pilot (18 months) preliminary cytogenetics/metaphase read protocol was instituted for 24- or 48-hour cultures with the goal of early reporting of MDS-associated and recurrent AML cytogenetics. This initial pilot included 1096 cases; 62 AML cases and 17 AML-MRC cases (10/17 i.e, 59% requiring cytogenetics for AML-MRC diagnosis). The average TAT was 2.4 and 9.12 days for the preliminary and final cytogenetic reports respectively. Based on preliminary cytogenetics, 5 patients were treated with Azacytidine with or without Venetoclax and one patient was treated with Vidaza; rest of the patients either received standard chemotherapy or hospice. 97.3% of cases had the same final report as the preliminary findings. Importantly, none of the abnormal preliminary findings

had to be rectified on final reporting. Only 29 cases (2.6%) had a discrepancy between a normal preliminary karyotype versus an abnormal final karyotype; these were all non-AML cases. Based on this promising data, this process was incorporated into the routine workflow. All cases with a myeloid indication that require a preliminary report have a 24- and 48-hour traditional cytogenetics culture established. Cultures are manually harvested, cell suspensions are dropped on slides, and stained using automated instrumentation. Subsequently, the slides from the 24-hour cultures are scanned using an automated metaphase scanning system and distributed to technologists who perform a five-cell metaphase analysis, the results of which are given to the cytogeneticist. We follow the College of American Pathologists and American College of Medical Genetics guidelines to define a clone i.e., presence of at least two cells containing the same extra chromosome(s) or structural chromosome abnormality or by the presence of at least three cells that have lost the same chromosome. The cytogeneticist interprets the findings and sends the preliminary report via secure email to the clinical and hematopathology team. To facilitate this change, the cytogenetics laboratory began utilizing the automated metaphase scanning system three times per day to ensure that slides needed for a preliminary analysis could be prioritized. Automation of the slide preparation and staining allowed these cases to be more efficiently scanned and this reduced slide preparation time from 6 to 0.5 minutes/slide. Currently, every bone marrow sample that comes through the laboratory receives a preliminary report based on the earliest diagnostically relevant culture (24 to 48-hour cultures for myeloid and 72-hour cultures for B/T-cell stimulated cultures).

To conclude, 62% of our cases needed cytogenetic studies to render a diagnosis of AML-MRC. The MDS FISH panel employed by most institutions typically includes EGR1 (5/5q-), D7S486 (7/7q-), CEP8 (+8), and D20S108 (20q). In the absence of an AML-MRC diagnosis, patients are put on the generic AML induction chemotherapy (7+3) and cannot be typically switched to CPX-351 later because of toxicity issues.[7] Therefore, it is crucial to have a

preliminary cytogenetic result within 2-3 days of an AML diagnosis to be able to accurately diagnose and treat most AML-MRC patients in a timely fashion. We demonstrate that a feasible option for generating rapid karyotype data is a preliminary conventional cytogenetics read on 24- and 48-hour cultures. While there are alternative promising assays available for generating karyotypic data e.g. chromosomal micro or "next generation" cytogenetics, including the use of whole genome sequencing or optical genome mapping, these might be not be able to provide information within 2-3 days of morphologic diagnosis of AML.[11-13] While most abnormalities would have been picked up by our FISH assay, 30% of cases had cytogenetic abnormalities which would have been missed on a routine MDS panel. In addition, FISH for AML and not MDS is typically ordered for AML; AML FISH panel varies between institutions and may or may not include probes relevant for AML-MRC diagnosis. An extended rapid FISH panel might serve the same purpose as preliminary conventional cytogenetics; however, it might come with additional logistical issues and cost. Considering that up to 1/3rd of AMLs are AML-MRCs, a timely diagnosis is crucial to ensure appropriate therapy for this relatively common AML subtype as well as for other AML subtypes. The recent incorporation of Gemtuzumab as frontline induction therapy for CD33+ AMLs, especially Core Binding Factor AMLs i.e., (t(8;21)(q22;q22) or inv(16) (p13q22)/t(16;16) also reinforces the need for rapid cytogenetics.[14,15] This can be achieved via a streamlined workflow in the cytogenetics laboratory and timely communication with the clinical and hematopathology teams. In conclusion, regardless of the technology, which might vary between institutions and between resource-rich and resource-poor countries, the recognition of a need to improve the karyotype turnaround time is of great importance.

AUTHORSHIP

AV, MM, KG and KI performed the research and data analysis, PK provided the clinical data and patient details and BS provided the cytogenetic raw data. AV, BS and MM wrote the manuscript. All authors read and approved the final manuscript.

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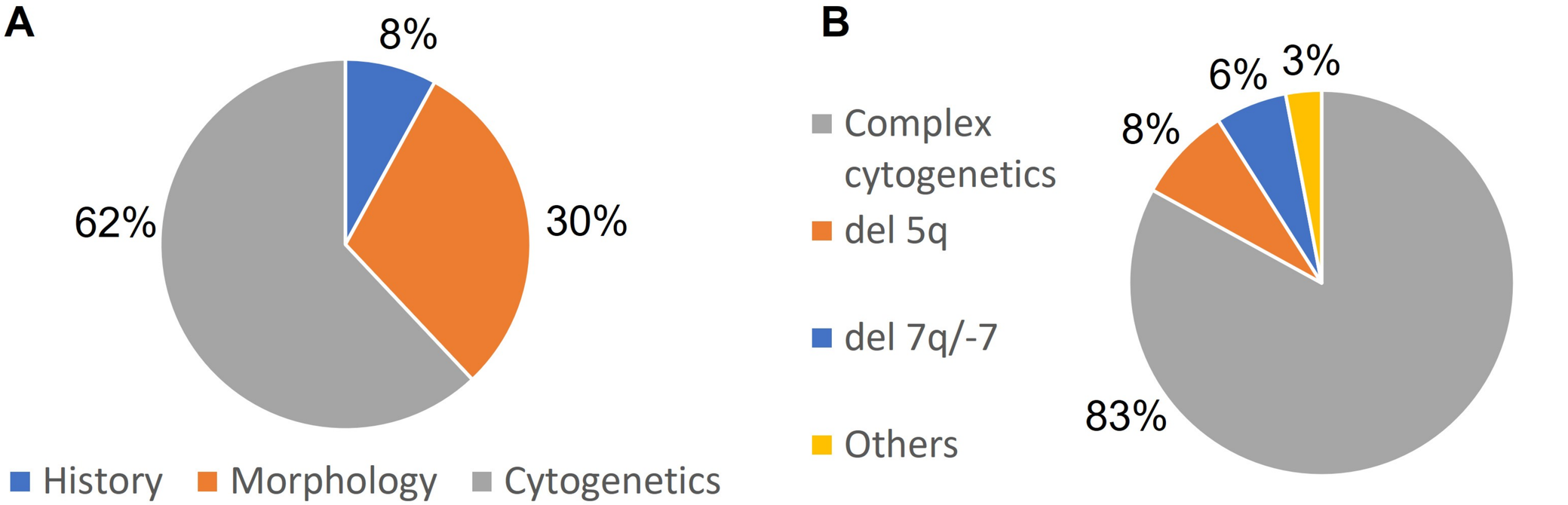
FIGURE 1

Figure 1. A) Percentage of cases diagnosed based on morphology, history or MDS associated cytogenetics B) Percentage distribution of MDS associated cytogenetic abnormalities C) Pseudo-pelger huet cell, 1000x D) dysplastic monolobated megakaryocytes

and megakaryocytes with separated nuclear lobes, 1000x E) Dysplastic multinucleated erythroid cell, 1000x

Figure 1


A
8%
62%
30%
History
Morphology
Cytogenetics
B
Complex cytogenetics
del 5q
del 7q/-7
Others
6%
3%
8%
83%
C
D
E