Case Report

Immunophenotyping, Cytogenetic and Mutational Analysis in newly diagnosed AML patient and corresponding laboratory investigations for therapeutic monitoring and prognosis: Case Report

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Abstract

Introduction

Traditionally, AML (Acute Myeloid Leukemia) classification and risk stratification was based on cytogenetic studies; however, molecular detection of gene mutations has achieved its growing role in classification, risk stratification, and management of AML. Current standard of care combines cytogenetic results with testing for mutations in FLT3, NPM1, CEBPA, and KIT to determine the prognostic subgroup.

Case presentation:

Here we present a case of AML of 75 years' male from western Nepal (Asian) who has improved symptoms and laboratory parameters after chemotherapy. Complete recovery from AML has also been reported earlier.

Conclusion:

Incorporation of new molecular markers to define prognosis in AML has helped to investigate the newer molecular targeting therapeutic agents.

Key words: AML, FLT3, NPM1, CEBPA, and KIT

Case report

Mr. Bhoj Raj Thapa (Name changed) the gentle man of age 75 years' from western Nepal (Asian) had cough for 6 weeks and fever for 3 days presented on 2016-08-12

On laboratory investigation (2016-08-14 to 2016-08-19)

His hemoglobin was 11.4, TC 68200, Platelets 78000, Blast cells 91% with auer rods.

LDH 1593 U/L (Normal: 313-618U/L)

Liver and Renal function test, Serum calcium and phosphorus, thyroid function test was all normal.

In, High resolution computed tomography(HRCT) of chest; multiple enlarged mediastinal nodes, minimal left sided pleural effusion and consolidation in upper lobe of left lung was noted.

On bone marrow examination 77.5 % blast cell were seen and acute leukemia was seen in biopsy.

In Flow cytometry, the blasts gated showed bright expression of HLA DR, CD 13, CD 33, CD 34 and CD 117 and dim expression of CD 7 and CD 64. Immunophenotyping demonstrated features of AML with monocytic differentiation

Mutational Analysis

FLT3 ITD (PCR and gel Electrophoresis): Not detected

NPM1 Mutational Analysis in Exon 12 of NPM1 gene (PCR and gene sequence): Not detected

Chromosomal Analysis (GTG Banding)

Non-availability of quality metaphase. Paucity of proliferating blast cells in the sample provided may be considered as possible reason.

Treatment

Patient was started on Azacytidine (Chemotherapy) and after 4 phases of first induction cycle chemotherapy, bone marrow examination report showed markedly hypercellular marrow with no morphological evidence of residual disease.

Discussion:

Previously AML classification done by French-American-British (FAB) was based on morphological and cytochemical staining properties. Todays WHO classification is based on morphology, immunophenotyping, cytogenetics and molecular studies that helps in better stratification and prognosis. In 2016, WHO revised its classification of myeloid neoplasms and acute leukemia, and it continues to define specific AML disease entities by focusing on significant cytogenetic and molecular subgroups.

Multiparametric flow cytometry is required to characterize myeloid neoplasm and analyze many cell in short time, characterizing many antigens per cell. The identification of leukocyte differentiation antigens allows for the detection of mixed, aberrant phenotypes and follow up of minimal residual cases. Ideally multicolor flow cytometry is used further to sub classify by lineage. The expression of certain antigens such as CD7, CD11b, CD14, CD56 and CD34 may be associated with adverse prognosis. In our case immunophenotyping was performed using cells labeled with antibodies for CD45, CD34, CD13, CD33, CD117, CD10, CD19, CD20, HLA-DR, CD3 and CD7 in a fresh bone marrow sample. Stain and lyse technique of whole blood was the method used and flow cytometry was performed (*4 color dual laser FACSCalibur, Becton Dickinson*). The blasts gated show bright expression of HLA DR, CD 13, CD 33, CD 34 and CD 117 and dim expression of CD 7 and CD 64. Immunophenotyping demonstrated features of AML with monocytic differentiation in this case.

Cytogenetic studies performed on bone marrow in patients with AML play a crucial role in characterizing the leukemia, helping determine disease aggressiveness, response to treatment, and prognosis. About 45% of acute leukemia have an abnormal karyotype with a recurrent chromosomal alteration, and about 15% have 3 or more cytogenetic abnormalities (Complex karyotype). (1)AML based on cytogenetics can be classified as favorable, intermediate and adverse risk group.

Table- Cytogenetic abnormality and risk groups

Risk Group	Cytogenetic Abnormality
Favorable	inv(16), t(16;16), t(8;21), t(15;17)
Intermediate	Normal cytogenetics, +8, t(9;11)
Adverse	t(6;9), inv(3), -7 monosomal karyotype, complex karyotype (≥3 chromosomal abnormalities)

WHO classification includes fusion genes in its classification, that are product of recurrent cytogenetic aberrations. Some of molecular abnormalities that are products of recurrent cytogenetic abnormalities incorporated by WHO in its classification are shown in table below.

Cytogenetic abnormality	Fusion genes
t(15;17)	PML-RARA (promyelocytic leukemia-retinoic acid receptor alpha)
t(8;21)	RUNX1-RUNX1T1 (Runt related transcription factor 1-Runt related transcription factor 1 translocated to 1)
inv(16) or t(16;16)	CBFB-MYH11 (Core-binding factor beta subunit- Myosin heavy chain 11 smooth muscle)
t(9;11)	MLLT3-MLL(Myeloid/lymphoid or mixed-lineage leukemia translocated to 3- Myeloid/lymphoid or mixed-lineage leukemia)

All the protein products of these fusion genes tend to block the hematopoietic cell differentiation.

In our case, cytogenetic study could not be carried out because of non-availability of quality metaphase. Paucity of proliferating blast cells in the sample provided may be considered as possible reason.

Many genetic abnormalities have been discovered in normal karyotype AML, especially mutation in

NPM1 (nucleophosmin), FLT3 (fms-related tyrosine kinase 3), CEBPA (CCAAT enhancer binding protein alpha), NRAS (Neuroblastoma RAS viral oncogene) BAALC (brain and acute leukemia gene), ERG (v-ets erythroblastosis virus E26 oncogene-like) genes etc. (2)Further, mutations in the genes that regulate *DNA* methylation, such as DNA methyltransferase 3 alpha (DNMT3A), Tet methylcytosine dioxygenase 2 (TET2), isocitrate dehydrogenase1 (IDH1)*, and* isocitrate dehydrogenase 2 (IDH2)has been suggested to have prognostic relevance in some cases of AML.

Out of these NPM1 ,FLT3 and CEBPA has established role in prognosis and has been included by WHO in AML classification.

FLT3(FMS related tyrosine kinase 3) is a member of class III receptor tyrosine kinase and plays an important role in regulating the proliferation, differentiation and survival of hematopoietic cells. Aberrant activation of receptor kinases is a common event in cancers, including leukemia. There are two common mutations that occur in *FLT3*:

a) An internal tandem duplication (ITD) in the juxta membrane domain in 20-35% and

b) A point mutation of the tyrosine kinase domain (TKD) in 5-8%. This involves point mutation of G to A at residue 835 that result in replacement of Aspartate (D) with Tyrosine (Y).

Both mutations lead to constitutive activation; however only the *FLT3* ITD is associated with a poorer prognosis.(3,4)*FLT3* ITD mutation is more common in AML with t(15;17). Identification of ITD mutation involves a PCR (Polymerase Chain Reaction) based test that can detect the larger PCR amplification products indicative of duplication.

Mutations in *NPM1*(Nucleophosmin)is the most frequent gene alteration in AML reported in 45-60% of normal karyotype.(3)Above 40 types of mutation is identified in NPM1 gene and most frequent is insertion or deletion of short oligonucleotides (4-10bp) at position 956 through 971 in exon 12 that lead to frameshift mutation in C-terminus of protein. A consequence of this mutation is that protein is unable to shuttle efficiently between nucleus and cytoplasm affecting ribosome biogenesis, centrosome duplication during mitosis, and cell proliferation and apoptosis through p53. Presence of this mutation confers better response to chemotherapy. Detection of NPM1 mutations typically involves a PCR assay that can distinguish the altered amplification products resulting from the nucleotide insertion or deletion mutation.

CEBPA (CCAAT Enhancer Binding Protein alpha) is a transcription factor involved in differentiation of granulocytes from hematopoietic precursors. *CEBPA* mutations are found in approximately 10% of AML and are more common in AML with a normal karyotype or with 9q deletions. *CEBPA* mutations in AML may be biallelic, which accounts for approximately two-thirds of cases, or monoallelic, accounting for the remaining cases. The type of mutation may be N-terminal frameshift mutation of protein coding regionleading to truncation of whole protein or C-terminal in-frame mutation that affect full length protein and short isoform which is normally coexpressed. In AML with a normal karyotype, isolated biallelic *CEBPA* mutations clearly confer a better prognosis, whereas a monoallelic mutation does not confer the same favorable prognosis.(5,6) Testing for CEBPA mutation requires detection of entire coding region so preferred method is DNA sequencing.

KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) is a receptor tyrosine kinase involved in proliferation, differentiation, and survival of hematopoietic cells. *KIT* mutations affect predominantly exons 8 or 17, lead to a gain of function and is reported in 12–25% of cases of core-binding factor (CBF)–AMLs such as inv(16)(p13q22) and t(8;21) AML.(7) This type of mutation is detected by highly sensitive mutation detection method known as mutation-biased PCR (MB-PCR).

Conclusion

Incorporation of new molecular markers in classification of AML will not only define the prognosis of patient but will also help to investigate the newer molecular targeting therapeutic agents that will alter the severity of disease. Moreover, research is going on to convert the AML cells into harmless macrophages. Also in future, we can use gene drive technology to destroy the brutality of leukemia cells.

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