REVIEW ARTICLE

Recent advances in diagnostic and prognostic aspects of acute myeloid leukaemia

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Abstract

Acute myeloid leukaemia (AML) is a group of haematological malignant disorders. Although not a new disease, many studies have been conducted to explore AML etiology, pathogenesis and prognosis at molecular level over the past two decades. A meticulous and continuous review of the available literature is still required to contemplate currently discovered information. We searched Google Scholar and PubMed by using different key word such as: updates in diagnostic criteria of AML, WHO classification of AML, new prognostic factors and risk stratification of AML. Mostly articles are referred from international sources published during last five years. Some older articles were only used when pivotal information required could not be surpassed by newer articles. Initially 50 relevant articles were included which were subsequently reduced to 36 by excluding articles with similar information. In this review an attempt is made to approach the subject in the light of currently available literature.

Keywords: AML, Acute myelogenous Leukaemia, Prognosis of AML, Diagnosis of AML.

Introduction

Acute myelogenous leukaemia (AML) is a condition characterized by malignant transformation of blood cells belonging to myeloid series that consists of granulocytes, monocytes, erythrocytes, and megakaryocytes. Thus, a combination of immature cells could be found in peripheral blood of such patients.[1]

Clinical Characteristics of AML

Patients of AML may present with a combination of various non-specific symptoms of unexplained weight loss, fever, thrombosis or anaemia, low immunity or blood loss. Similarly, the physical examination may reveal nonspecific findings such as pallor, lymph node enlargement, hepatomegaly and splenomegaly.[2]

Large number of studies regarding the karyotyping and gene abnormalities has enabled a clearer understanding of AML pathology during the last few decades. This has also helped in predicting the prognosis and tailoring therapy. Despite the surge of knowledge, morphology of bone marrow and blood is still the goldstone of diagnosis. The FAB classification of AML was recommended by French-American-British Cooperative Group. It is based on number of blast cells and partially or fully differentiated mature cell type on smear of the blood and/or bone marrow sample. FAB classification has been used for many years because of the availability of peripheral blood or bone marrow aspirate samples in routine. The minimum requirement for the FAB classification is Romanowsky smear and myeloperoxidase reaction test. In addition some cytochemical staining procedures such as Sudan black B, Periodic acid -Sciff (PAS), esterases and acid phosphatases were also used and were helpful. The cut off for abnormal cells on morphology was 50%, to differentiate it from dysmyelopoietic states that remained static for long time.[3]

The FAB group classified AML into six types as given in Table-1.

In 1988 a set of recommendations were developed for clinical trials in AML that was used to design and report AML clinical trials at the National Cancer institute in United States. In 2001, revised recommendations were devised due to further research in AML biology and genetics, in which de novo AML was defined as patient with no clinical history of prior myelodysplastic syndrome (MDS), myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents. Secondary AML was defined as patients who have a clinical history of myelodysplastic syndrome (MDS), myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents and should be further categorized as AML secondary to an already existing specific situation. A history of fatigue, bleeding, or recurrent infections that preceded the diagnosis of AML by 1 month or greater, although suggestive of a preleukemic state, should not by itself allow designation of a case in this category without confirmation of an existing peripheral blood film that demonstrates morphologic dysplasia.[4]

A new classification of AML was developed by the joint

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Table-1: FAB Classification of AML. (Bennet et al 1976)

French-American-British (FAB) Classification of AML		
FAB Subtype	**Dominant Morphology**	**Comments**
M0	Undifferentiated	Myeloperoxidase negative
M1	Myeloblastic without maturation	Some evidence of granulocyte differentiation
M2	Myeloblastic with maturation	Maturation at or beyond the promyelocytic stage of differentiation; can be divided into those with t(8;21) AML-ETO fusion and those without
M3	Promyelocytic	APL; most cases have t(15;17) PML-RAR or another translocation involving RAR
M4	Myelomonocytic	
M4	Myelomonocytic with bone marrow eosinophilia	Characterized by insertion of chromosome 16 involving CBF , which normally forms a heterodimer with AML1
M5	Monocytic	
M6	Erythroleukaemia	
M7	Megakaryoblastic	GATA mutation associated with Down's syndrome

Table-2: Classification of AML.

Revised WHO myeloid neoplasm and acute leukaemia classification - 2016[6]
Acute myeloid leukaemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8; 21)(q22; q22.1); RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16; 16)(p13.1; q22); CBFB-MYH11
APL with PML-RARA
AML with t(9; 11)(p21.3; q23.3); MLLT3-KMT2A
AML with t(6; 9)(p23; q34.1); DEK-NUP214
AML with inv(3)(q21.3q26.2) or t(3; 3)(q21.3; q26.2); GATA2, MECOM
AML (megakaryoblastic) with t(1; 22)(p13.3; q13.3); RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukaemia
Acute monoblastic/monocytic leukaemia
Pure erythroid leukaemia
Acute megakaryoblastic leukaemia
Acute basophilic leukaemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukaemia associated with Down syndrome
Blasticplasmacytoid dendritic cell neoplasm
Acute leukaemias of ambiguous lineage
Acute undifferentiated leukaemia
Mixed phenotype acute leukaemia (MPAL) with t(9; 22)(q34.1; q11.2); BCR-ABL1
MPAL with t(v; 11q23.3); KMT2A rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

efforts of World Health Organization (WHO), Society for Haematopathology and the European Association of Haematology. WHO classification divides AML to some extent into groups that have favourable, intermediate and adverse outcome based on the presence of recurrent genetic abnormalities, multilineage dysplasia, and otherwise categorized.[3]

Criterion for diagnosing AML was revisited in 2008 to include the effect of cytogenetics and molecular abnormalities. The percentage of blast cells and abnormal cells should be determined after staining with on May-Grunwald-Giemsa or a Wright-Giemsa. Counting of 200 leukocytes on peripheral blood smear and 500 nucleated cells on aspirate smear should be done.[4,5] The cut off line of blast percentage in both blood and marrow, is >20% instead of 30% for the diagnosis of AML. Thus, the MDS category of "refractory anaemia with excess blasts in transformation" is eliminated as a separate entity. The clonal, recurrence of cytogenetic abnormalities t(8;21)(q22;q22), inv(16)(p13q22) or (16;16)(p13;q22), and t(15;17)(q22;q12) is AML regardless of blast cells percentage.[4] In t(9;11)(p22;q23) or other 11q23 abnormalities 20% or more blast cells should be present to label it as AML.[3] t(9;11)(p22;q23) MLLT3-MLL is regarded a separate category according to WHO classification.[5] Recently, an updated WHO classification[6] was published (Table-2).

In cytochemistry, both peroxidase and esterase are of value. The presence of myeloperoxidase (MPO) with >3% blasts confirms myeloid lineage. Additionally, lineage can be evaluated by immunophenotyping of expression markers in 10-20% leukaemic cells.[4]

Prognostic Factors

General Characteristic

General prognostic factors may be patient related, for

example, age and occupation or disease related, such as morphology and blast cell count.

Age is one of the unfavourable prognostic factors that should be taken into account while treating AML. Older AML patients show less favourable and more adverse cytogenetic abnormalities. For example, Röllig et al. (2011) reported that in AML patients aged <60 years Complete Remission (CR) rate was 76% as compared to 51% in those>60 years with gradual decline with increasing age at the diagnosis.[7]

A day-16 blast number is another prognostic indicators observed by German AML Cooperative Group (AMLCG). If blast number is less than 10% at day 16 after start of induction, the prognosis was found favourable while in those cases where blasts were above 10% the event free and survival rates were low.[8]

White blood cell (WBC) index is the product of WBC count by the ratio of marrow blast. It was found as an indicator of disease prognosis in French multicenter AML trial in AML with t(8;21) (equivalent to M2 type in FAB classification). Three groups identified were low WBC index of 2.5, intermediate index of 2.5-20 and high index of 20 or more. Higher index was associated with lower CR duration as well as disease-free and overall survival rates.[9]

Secondary AML (s-AML) group has prior history of MDS or myeloproliferative neoplasm (MPN) or therapy related AML (t-AML). It is also observed that t-AML has different outcome depending on the chemotherapeutic agent used and the stage of AML at the time of diagnosis. s-AML with a prior MDS or MPN has a poor prognosis while s-AML with MPN may be worse as compared to s-AML with prior MDS and t-AML.[10]

Cytogenetic Factors and ELN Guidelines

Cytogenetics is an important tool for predicting the prognosis of AML. Many mutations and karyotyping abnormalities are associated with AML prognosis. These genetic abnormalities include gene amplification, point mutation, insertion, deletion, polymorphism and unregulated expression. European Leukaemia Net (ELN) has graded AML into favourable, intermediate and adverse groups according to the prognosis that is associated with reported translocations and mutations (Table-3). ELN stratification is found consistent with the outcome by some[11] while others consider it feasible for AML in young age and recommend further stratification of the diagnostic criteria for older age group. Gene mutations and expression levels can be used as an adjunct to the karyotyping to improve risk stratification, understanding and management of AML patients.[7]

Karyotypes related to outcome

Karyotypes related to Favorable outcome

Core binding factor (CBF) AML

CBFs are four different transcription factors, three DNA binding subunits runt-related transcription factor 1 (Runx1), Runx2, or Runx3 and one non-DNA binding CBF subunit that stabilizes the binding of the subunit to the DNA. RUNX1 and CBFβ are essential for normal haematopoiesis and required for cellular differentiation. Disorders of the RUNX1or CBFβ considered to be involved in development of AML are results of gene rearrangement in case of translocations. The fused proteins from these genes are thought to be involved in the pathogenesis of AML. CBF- AMLs may either have t(8;21)(q22;q22); RUNX1-RUNX1T1 inv(16)(p13.1q22), t(16;16)(p13.1;q22) or CBFB-MYH11. Patients with these anomalies have shown higher CR rate and better OS than patients with NPM1 or CEBPA mutations with normal karyotype and without Flt3-ITD mutation (5,7). Gene rearrangements such as RUNX1-RUNX1T1, CBFB-MYH11 can be analyzed by FISH or by RT-PCR.[5]

Acute Promyelocytic Leukaemia (APL) and Translocation (15;17) in APL

APL also called M3 type in FAB classification has different

Table-3: ELN Guideline for Cytogenetic Classification.[7]

ELN Genetic Risk Group	Subsets
Favourable	t(8;21)(q22;q22); RUNX1-RUNX1T1inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
	Mutated NPM1 without FLT3-ITD (normal karyotype)
	Mutated CEBP?(normal karyotype)
Intermediate-I	Mutated NPM1 and FLT3-ITD (normal karyotype)
	Wild-type NPM1 and FLT3-ITD (normal karyotype)
	Wild-type NPM1 without FLT3-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); MLLT3-MLL Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1t(6;9)(p23;q34); DEK-NUP214t(v;11)(v;q23); MLL rearranged?5 or del(5q); ?7; abnl(17p); complex karyotype

Abbreviation: ELN, European LeukaemiaNet.

cytogenetics and pathophysiology. A specific translocation of t(15;17) is found in all patient of APL. Retinoic acid receptor (RAR) gene on chromosome 17 is fused to another gene on chromosome 15 resulting in a chimeric protein formation which inhibits the process of cell maturation at some point during haematopoiesis resulting in APL. APL has good treatment outcome and overall survival.[12]

Karyotypes related to Intermediate Prognostic Outcome

This category includes patients with karyotyping that fits neither favourable nor adverse prognosis group. Cytogenetically normal AML have intermediate prognosis and can benefit from stem cell transplantation. In addition AML with t(9;11)(p22;q23); MLLT3-Myeloid/Lymphoid or Mixed-Lineage Leukaemia (MLL) (Röllig et al. 2011[7]), +8, +21, +22, del(9q), del(7q) are also found associated with intermediate prognosis in terms of CR, relapse and overall survival rates.[3]

Karyotypes related to Adverse Prognostic Outcome in AML

Complex (multiple) karyotypes -5, del(5q), -7, inv(3), abnormalities of 3q, t(3;3), found mostly in higher age group have worst prognosis.[3,7,13] The prognosis is worst in those who have two or more monosomies (-5, -7, -17) or one monosomywith a structural chromosomal anomaly (inv(3), t(3;3), and del(5q)).[13]

MLL gene (also called lysine methyltransferase 2A and KMT2A) located on chromosome 11q23encodes a transcription factor that methylates the histones of DNA thus regulating the gene expression during foetal development and haematopoiesis. Many translocations such as t(15;17), t(8;21) or inv(16)/t(16;16), (5q_/5, 7q_/_7, inv(3)/t(3;3), 12p_, 17p abnormalities, and complex karyotypes reportedly related with MLL are involved in the pathogenesis in both AML and acute lymphoblastic leukaemia (ALL). Most of the MLL related translocations are associated with poor prognosis in both paediatric and adult AML[14] while some types have relatively favourable outcome when treated with high dose during induction.[15]

Gene Mutations

Gene mutations are found correlated with both diagnosis as well as prognosis of AML. Both WHO and ELN classification have taken these mutations into account to stratify AML patient according to their predicted outcome of chemotherapy and bone marrow transplant.

Flt3 Mutation

Flt3 (CD 135) is a receptor with FMS-like tyrosine kinase 3 (Flt3) activity. It is expressed in early haematopoietic progenitor cells but not in mature differentiated cells. The ligand Flt3l acts as growth hormone playing role in stem cell survival and differentiation.[16] Mutation in the gene of Flt3 especially internal tandem repeats (ITD), leads to constitutive activation causing auto-phosphorylation and phosphorylation of several other proteins inside the cell. Thus some genes are up-regulated and others are down regulated causing blockade in differentiation.[16] Frequency of FLT3/ITD mutation is 30-40% in adult AML patients with normal karyotyping. Four year survival rate of patients with only FLT3 mutation is 20-25%.[3] AML of intermediate-risk with Flt3-ITD show reduced OS. Integrated gene studies have shown that AML of intermediate-risk with Flt3-ITD mutation have better prognosis if found along with CEPBA mutation and worse prognosis when found along with TET2, DNMT3a and trisomy 8.[15]

Nucleophosmin 1 (NPM1)

NPM1 which is also called B23 is a nucleo-cytoplasmic shuttling protein that is coded by a gene on chromosome 5q35. It is involved in ribosome biogenesis, chromatin remodeling, mitosis, DNA repair, replication, transcription and p53 pathway. Its role as a histone chaperone may be responsible for many of its functions. NPM1 mutation is found in 50-60% cytogenetically normal AML patients and four year survival rate is 50%.[3]

NPM1 and Flt3 mutations frequently coexist. AML with NPM1 mutation without FLT3 mutation have a good prognosis. However, the reverse has worse prognosis and if they coexist, its 'intermediate' prognosis.[17] Patients with NPM1 have better high dose chemotherapy outcome and its OS. Coexistence of NPM1 and Isocitrate dehydrogenase-1 (IDH1) or IDH2 mutations is also favorable for treatment with high dose chemotherapy.[15]

CCAAT enhancer binding protein (C/EBP)

CEBPA or C/EBP is a transcription factor favouring granulocyte differentiation. Two types of mutations have been reported in C/EBP protein AML other than APL. One mutation is found in C-terminal of C/EBP protein that is part of leucine zipper domain, thus diminishing dimerization and DNA binding. The second mutation at N-terminus renders it nonfunctional. Patients with double mutation of C/EBP have better prognosis than with single or no mutation in terms of CR and survival rates, even in the absence of Flt3/ITD mutation.[18] CEBPA mutation alone is found in 15% of AML patients with normal karyotyping and such patients have a 4-year survival rate up to 60%.[3]

Ten-Elevan-Translocation-2 (TET2) Mutation

TET2 gene located on chromosome 4q24 codes for an enzyme that converts methylcytosine to 5-hydroxymethylcytosine (5hmc) required for normal DNA activation. TET2 mutation is a recent finding that should be added to ELN classification to improve risk stratification TET2 mutation is found as an adverse prognostic factor even if it coexists with favourable[19] or intermediate-risk cytogenetics.[15] Adversity of TET mutation become higher in the presence of FLT3-ITD, NPM1 negative, or poor prognostic genotypes but not in FLT3-ITD negative patients andNPM1 and or CEBPA positive AML.[20] Overall survival is reduced with TET mutation.[15] However, others have shown no correlation of TET2 with AML prognosis.[21]

DNA (Cytosine-5-) Methyltransferase 3 Alpha (DNMT3A Gene)

DNMT enzymes methylate the unmethylated CpG island on DNA to regulate gene expression. DNMT3a is involved in embryonic development, imprinting, and X-chromosome inactivation. DNMT3a gene mutation is found more frequently in M4 and M5 types (FAB classification) rendering adverse prognosis in AML.[15] DNMT3a gene mutation affects the prognosis adversely in older AML patients.[22] Contradictory results have been shown regarding the association of DNMT3a mutation and AML prognosis. Dose intensive induction chemotherapy is associated with better out come in DNMT3a mutant patients as compared to wild type. Treatment with higher dose of daunorubicin (90mg/m^2) is found associated with better outcome as compared to lower dose (45mg/m^2) along with cytarabine in DNMT3a mutant AML patients when compared with wild type DNMT3a.[15]

Wilms' Tumour 1 (WT1) Gene Mutation

WT1 is a gene that codes for a zinc-finger transcription factor. It is physiologically expressed in embryonic kidney cells and haematopoietic stem cells. Numerous WT1 mutations are reported. WT1 mutation is reported as an independent risk factor for lower CR, OS and DFS in AML patients, especially with normal karyotype.[23]

c-Kit gene Mutation

c-kit gene encodes for a proto-oncogene that encodes a receptor for stem cell factor with tyrosine kinase activity. It regulates cell survival and proliferation and stem cell maintenance during haematopoiesis. Mutation of this gene is correlated with reduced overall survival in AML patients with t(8;21) but not with abnormalities of chromosome 16.[15] c-Kit mutations should be included in risk stratification.

Isocitrate Dehydrogenate (IDH) 1 and 2

IDH catalyzes decarboxylation of isocitrateto alpha-ketoglutarate (α-KG) and is found in humans in three isoforms IDH1, IDH2 and IDH3. IDH1 found in cytosol and IDH2 found in mitochondria. The genes for IDH1 and IDH2 are located on chromosomes 2 and 15 respectively. IDH3 is involved in citric acid cycle. IDH1 and 2 reduces the oxidative stress of the cell by providing NADPH for NADPH-dependent antioxidant enzymes. Normally IDH1 and 2 converts isocitrate to α-KG (α-KG) but the mutated forms reduce α-KG to a new compound 2-hydroxyglutarate (2-HG), an oncoprotein involved in pathogenesis of leukaemia. High serum 2-HG levels predict poor prognosis.[24] One of the mechanisms found in IDH1/2 mutation is increased expression of anti-apoptotic protein BCL-2 by the oncoprotein 2-HG. This is also supported by the trials of anti BCL-2 agent ABT-199 in IDH 1/2 mutant AML.[25] Both IDH1 and 2 mutations are associated with poor prognosis in AML.IDH1 mutations are more frequently found in cytogenetically normal AML correlated with lower CR rates.[26] AML patients having both NPM1 and IDH1 and/or IDH2 mutation show better prognosis as compared to those with no mutation.[15]

Gene Expression Levels

Multidrug-resistance 1(MDR1) protein

ABC (ATP-binding cassette) transporters are a group of membrane transporters. ABC transporters transport various substances across the biological membranes by hydrolyzing ATP to ADP. Among this family, ABC1 transporter, also called multidrug resistance protein 1 (MDR1) or Permeability-glycoprotein (P-pg), is responsible for efflux of various foreign substances, toxins and drug molecules (including chemotherapeutic agents) from the cell. Hence, MDR1 overexpression in leukaemic cells is associated with lower CR rate and higher relapsed rate in acute leukaemia, especially in old age.[27]

Multidrug resistance associated protein 1&2 (MRP 1 & 2) and Lung Resistance related Protein (LRP)

MRP1a member of ABCC (ATP binding cassette C) transporter family, is similarly involved in transportation of different substances including anticancer drugs. Overexpression of MRP2 can lead to drug resistance by decreasing its accumulation inside the cell. This resistance can be overcome by using inhibitors of MRP2, for example curcumin.[28] Likewise, overexpression of LRP leads to lower CR rate in AML.[27]

Table-4: Prognostic Factors of AML based on discussion in the current article.

#	Prognostic factors	Value	Favorable Prognosis	Unfavorable Prognosis	Study
1. General Characteristic					
i.	Age	>60 years	-	Yes	Röllig et al. 2011[7]
ii.	Day 16 blast count	>10%	-	Yes	Kern et al. 2003[8]
iii.	WBC index (WBC count ´marrow blast)	10-27.5	-	Yes	Nguyen et al. 2002[9]
iv.	Secondary AML with MDS and MPN	-	-	Yes	Szotkowski et al. 2010[10]
2. Cytogenetic factors & ELN guidelines					
Karyotypes: Chromosomal anomalies and gene rearrangement:					
1	Favorable karyotypes				
	Core binding factor (CBF) –AML	-	Yes	-	Dohner et al 2010[5]
					Röllig et al. 2011[7]
	Acute promyelocytic leukaemia (APL) and t(15;17) in APL	-	Yes	-	Kamimura et al. 2011[12]
2	Karyotypes related to Intermediate Prognostic outcome				
	AML with t(9;11)(p22;q23); MLLT3-MLL	-		Yes (Intermediate)	Röllig et al. 2011[7]
	+8, +21, +22, del(9q), del(7q)				Vardiman et al. 2009[3]
3	Karyotypes related to Adverse Prognostic outcome in AML:				
	AML with complex karyotyping -5, del(5q), -7	-	-	Yes	Vardiman et al. 2009,[3]
					Medeiros et al. 2010,[13]
	MLL related translocations				Röllig et al. 2011[7]
	More than one monosomy: -5, del(5q), -7, -17	-	-	Yes	Medeiros et al. 2010[13]
	One monosomy and inv(3) of 3q, t(3;3) and del(5q)				Meyer et al. 2013[14]
Gene Mutations					
1. Flt3 mutation		-	-	Yes	Small D. 2006[16]
					Vardiman et al. 2009[3]
					Patel et al. 2012[15]
2.	Nucleophosmin 1 (NPM1) mutation	-	Yes	-	Vardiman et al. 2009[3]
					Patel et al. 2012[15]
					Liu et al. 2013[17]
3. CCAAT enhancer binding protein a (C/EBPa)		-	Yes	-	Vardiman et al. 2009[3]
					Green et al. 2010[18]
4. Ten-Elevan-Translocation-2(TET2) Mutation (Homozygous)		-	-	Yes	Metzeler et al. 2011[19]
					Chou et al. 2011[20]
					Gaidzik et al. 2012[21]
					Patel et al. 2012[15]
5.	DNA (Cytosine-5-)-Methyltransferase 3 Alpha (DNMT3A Gene)	-	-	Yes	Patel et al. 2012[15]
					Ostronoff et al. 2013[22]
6. Wilms' Tumor 1 (WT1) gene mutation		-	-	Yes	Aref et al. 2014[23]
7. C-Kit gene Mutation		-	-	Yes	Patel et al. 2012[15]
8. Isocitrate Dehydrogenate (IDH) 1 & 2		-	-	Yes	Patel et al. 2012[15]
					Feng et al. 2012[26]
					Wang et al. 2013[24]
					Chan et al. 2015[25]
Gene Expression Levels					
		Expression			
1. Multidrug-resistance 1(MDR1)		↑	-	Yes	Tsuji et al. 2012[27]
2. Multidrug resistance associated protein 1 & 2 (MRP1 &2)		-	-	Yes	Wortelboer et al. 2003[28]
3. BCRP and LRP		↑	-	Yes	Tsuji et al. 2012[27]
4. Deoxycytidine kinase (dCK)		↑	Yes	-	Yamauchi et al. 2009[29]
1. Topoisomerase II α & β		↑	-	Yes	Wang et al. 2009[31]
					Pommier et al 2010[30]
2. BCL-2 & Bax		↑	-	Yes	Del Poeta et al. 2003[34]
					Fluda and Debatin, 2006[33]
					Reubold et al. 2012[32]
3. Wilms' Tumor 1 (WT1) gene Expression		↑	-	Yes	Lyu et al. 2014[35]
					Gray et al. 2012[36]

Deoxycytidine Kinase (DCK)

DCK is an enzyme that phosphorylates purines (deoxyadenosine and deoxygaunosine) and a pyrimidine (deoxycytidine) as well as their analogues used as chemotherapeutic agents. Lower expression of dCK gene has been observed in Ara-C resistant AML cases. However, ratio of dCK and cytosolic 5'-nucleotidase II (cN-II), which inactivates Ara-C by dephosphorylation, has been shown to predict ara-C sensitivity.[29]

Topoisomerase II α and β

During normal replication or transcription, DNA double helix should unwind to allow the enzymes and other proteins involved in this process. Unwinding leads to positive and negative DNA supercoils. Topoisomerases reduce these supercoils by cutting and re-ligating either single or both strands. Topoisomerase (TOPO) IIα and IIβ are the enzymes in humans that can cut and ligate double strands. Etoposide, an anticancer drug binds with topoisomerase-II and prevents re-ligation of DNA strands. Anticancer drug doxorubicin and other DNA intercalating agents, also inhibit topoisomerase II to ligate the cut DNA strands. Hence, topoisomerase II expression has been investigated in terms of clinical outcomes.[30] A change in the Topo IIα mRNA level could predict a bad response to treatment. AML patients with increased expression of Topo IIα mRNA levels at relapse do not respond to second induction.[31]

Bax and Bcl-2 in AML

Apoptosis or programmed cell death is required to get rid of the damaged cells to ward off aberrant growth of normal tissues into a neoplasm. Many anticancer drugs ultimately lead to apoptosis in malignant cells as part of their mode of action. The process of apoptosis is highly regulated. Bcl-2 protein in the cytoplasm acts as inhibitor of apoptosis while Bax promotes apoptosis.[32]

Defects in apoptosis and overexpression of Bcl-2 have been found associated with malignant transformation as well as poor response to chemotherapy and indicates poor prognosis.[33] A lower Bax/Bcl-2 ratio is associated with chemoresistance and adverse cytogenetics in AML. A higher Bax/Bcl-2 ratio has been found to correlate with higher CR rate and hence a better outcome.[34]

Wilms' Tumor 1 (WT1) gene Expression in AML

WT1 already described in the previous section, is expressed in early stages of haematopoiesis. In normal karyotype AML, overexpression of WT1 at diagnosis has been associated with decreased CR, DFS and OS in both Flt3 negative and Flt3 positive AML patients.[35] Additionally higher blood and marrow WT1 levels at diagnosis and post consolidation correlates with higher risk of relapse.[36]

A comprehensive analysis of the discussion is given in Table-4. In conclusion it is clear from the above discussion that pathogenesis of AML is a complex multifactorial process with a heterogeneous acquired background. Detailed characterization of inherited and acquired genetic variants in AML helps to predict prognosis and treatment strategies aiming at improving outcome. Thus, AML prognosis and outcome not only depends upon the factors related to disease itself but also upon the factors that alter drug sensitivity. Hence, a combination of these factors could be helpful to achieve a better outcome.

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