Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2019, 8(6): 135-142

Hemoglobin Level and Karyotype Status are Independent Prognostic Parameters for Acute Myeloid Leukemia: Pilot Study of 244 Patients

Manoj Kumar[1,2#], Vikas Gaur[2#], Mohit Chowdhry[1], Raj Nath Makroo[1,3] and Pankaj Sharma[2]*

[1] *Department of Molecular Biology and Immunology, Indraprastha Apollo Hospitals, New Delhi, India*

[2] *Laboratory of Oxidative Stress and Cancer Biology, Centre for Medical Biotechnology, Amity Institute of Biotechnology, Amity University Uttar Pradesh, India*

[3] *Department of Transfusion Medicine, Rockland Group of Hospitals, New Delhi, India*

[#] *Contributed equally to the work*

**Corresponding e-mail: psharma8@amity.edu*

ABSTRACT

Purpose: *Acute myeloid leukemia (AML) is a heterogeneous disease with genetic profiling being the primary prognostic factor. The objective of this study was to examine if routinely acquired parameters may be used to improve the prognosis of AML prognosis.* ***Methods:*** *Karyotyping was performed using bone marrow-derived mononuclear cells of 244 de novo diagnosed AML patients and age, sex, total leukocyte count (TLC), platelet count and hemoglobin (Hb) levels at initial presentation were recorded. The patients were given standard treatment and overall survival (OS) for 1 year and 5 years were recorded.* ***Results:*** *As expected, patients with aberrant karyotype status had poor overall survival. Aneuploidy was strongly associated with poor patient survival; while patients presented with hyperploidy had significantly lower OS at both 1 year and 5 years of time points; hypoploidy was correlated only with poor 1 year OS. Interestingly, 146 patients with Hb levels ≤ 8 g/dl had significantly lower 1 year and 5 years OS compared to 95 patients with Hb levels ≥ 8 g/dl. Combining karyotype status or Hb levels with other parameters did not improve patient prognosis.* ***Conclusion:*** *In summary, our results show that in addition to karyotype status, Hb level is an independent prognostic marker that should also be considered for early identification of patients that may benefit from alternative therapies.*

Keywords: Acute leukemia, Prognosis, Karyotype, Hemoglobin levels, Overall survival

INTRODUCTION

Acute myeloid leukemia (AML) is a clonal disorder arising from the malignant transformation of the myeloid progenitor cells at different stages of differentiation, leading to accumulation of immature, non-functional blast cells in the bone marrow and in peripheral blood [1]. It is diagnosed on the basis of the analysis of bone marrow and peripheral blood; analytical parameters include cell morphology, immunophenotyping based on both cell surface and cytoplasmic markers [2], and cytogenetic (both conventional and molecular) [3]. Nevertheless, specific genetic lesions, detected using either conventional cytogenetics or molecular methods, form the primary basis of the updated WHO classification for AML [4]. Treatment for AML has remained the same for the last 30-40 years with minor updated recommendations [5,6]. Intensive induction therapy remains the mainstay of primary treatment for patients <60 years of age followed by consolidation therapy if complete disease remission is achieved, though it has been suggested that healthy older patients may benefit more from intensive rather than non-intensive induction therapy [7]. Post-remission therapies include chemotherapy followed by autologous or allogeneic hematopoietic cell transplantation (HCT) [6]. Patients are unable to tolerate intensive chemotherapy are provided with supportive care, low-dose chemotherapy or investigational drugs. Novel targeted drugs being actively investigated are inhibitors targeting tyrosine kinases, topoisomerase II and epigenetic modifiers [8-13]. Immunotherapy, adoptive cell transfer approaches, and cell cycle checkpoint inhibitors hold a lot of promise for patients not suitable for intensive therapy, ineligible for HCT or with

residual disease following intensive induction therapy [14]. Gemtuzumab ozogamicin (GO) is an anti-CD33 antibody which has recently been approved by the US FDA for treatment of relapsed/refractory CD-33 positive AML patients older than 2 years of age [15].

Though the parameters for the diagnosis of AML are well-defined [4], factors for predicting disease outcome are variable and may be broadly classified as patient-associated and disease-related. While patient-related factors affecting AML prognosis include age, mode of induction therapy (intensive vs. non-intensive), performance status and general health status of patients like specific co-morbidities [16-18], disease-related factors include clinical parameters (for e.g., cell counts and Hb levels) and specific genetic lesions which remain the strongest prognostic factors [19-22]. Even with a reasonable understanding of the factors influencing treatment outcomes for AML patients, the ability to predict disease prognosis remains limited [23], probably due to changes in the landscape of genetic lesions with age and also due to minor factors that may influence disease prognosis [16,23,24]. Recent studies have demonstrated that taking multiple factors (both patient- and disease-related) into account can significantly improve prognostic efficiency [19,21,25-27].

In the current study, we assessed 1 year and 5 years overall survival (OS) for 244 newly diagnosed AML patients and looked at their correlation with patient-related factors such as age and sex with disease-related factors such as total leukocyte count (TLC), platelets count, hemoglobin (Hb) levels and cytogenetic status. Individually, cytogenetic status and Hb levels could predict 1 year and 5 years OS. Among karyotypic abnormalities, aneuploidy had the most significant effect on patient survival with hyperploidy having a more pronounced effect than hypoploidy. In summary, our study suggests that in addition to the well-established role of aneuploidy, Hb level is an easily accessible, independent parameter that may also be used as a prognostic marker for AML.

MATERIALS AND METHODS

Patient Population

The study initially included 342 patients newly diagnosed with AML at Department of Molecular Biology and Transplant Immunology, Apollo Hospital, New Delhi between December 2009 and December 2012 and consented to be a part of the study. The study was approved by the Institutional Ethics Committee. Informed consent was obtained from all individual participants included in the study. Individuals already undergoing treatment for AML and patients with secondary AML after been diagnosed earlier with Down's syndrome or myelodysplastic syndrome were excluded from the study. At the end of the study, 244 patients could be followed up for 5 years. All clinical parameters including total leukocyte count, platelet count, and hemoglobin levels were analyzed using hematological analyzer (ADVIA 21201 hematology System). The diagnosis of AML was done according to 2008 WHO guidelines with the incorporation of morphology, cytochemistry, immunophenotype, cytogenetics and clinical data [28].

All patients were provided standard care and treatment was initiated as per clinical recommendations. Majority of patients (212-86.9%) were put under intensive induction chemotherapy, others were either given allogeneic HCT (27-11.1%) or initiated azacitidine therapy (5-2%).

Karyotype Analysis

Heparinized bone marrow and/or peripheral blood samples were collected in syringes or test tubes and were sent to the laboratory at room temperature. Samples without anticoagulant or with EDTA were classified as unsuitable and were excluded from the analysis. Two different cultures (for 24-hour culture, and 48-hour culture) were prepared from these samples using RPMI 1640 medium supplemented with 20% FCS (fetal calf serum), L-glutamine and penicillin/streptomycin (50 IU/ml and 50 µg/ml respectively). Metaphases were harvested by adding colcemid (10µg/ml) solution followed by hypotonic KCl (0.075 M) treatment and fixation using a standard 3:1 methanol: glacial acetic acid fixator. The fixed slides were used for conventional karyotype analysis using the conventional Giemsa banding (GTG-banding) technique according to the International System for Human Cytogenetic Nomenclature (ISCN 1995) [29,30]. Five to ten slides were screened in each case and 10-20 metaphases were analyzed for each sample. The analysis was carried out using a BX51 Olympus microscope and images captured with an automated image analysis system (Cytovision, Applied Imaging).

Statistical Analysis

Patient survival, calculated for the period of 1 year and 5 years from the date of enrolment, was the primary end-point of the study. The correlation of patient survival with individual parameters was estimated using the log-rank Mantel-Cox test; multivariate analysis was performed using the stepwise Cox proportional hazard regression method. The data was analyzed using SPSS version 23.

RESULTS

The characteristic features of the study population with their clinical parameters are summarized in Table 1. Of the 244 AML patients, 162 patients (66.4%) were males and 82 patients (33.6%) were females. The mean age of patients was 39.03 years with a range of 1-89 years, majority (83.6%) of them were above 18 years of age. Similarly, a majority of the patients had TLC ≤ 50000 cells/cumm (71.7%), platelet count ≤ 50000 cells/cumm (58.6%) and Hb levels ≤ 8 g/dl (59.8%). The 1 year and 5 years of survival rates were 46.3% and 36.5% respectively and were significantly correlated with Hb levels only; Hb levels ≤ 8 g/dl was associated with poor survival (Table 1).

Table 1 Baseline characteristics of 244 AML patients at initial diagnosis and their correlation with 1 year and 5 years of overall survival (OS)

Clinical parameters	Patient No.*	% OS		p-value	
		1-Year (46.31%)	5-Year (36.47%)	1 Year OS	5 Year OS
Sex					
Male	162 (66.39%)	46.29	37.65	0.9384	0.5915
Female	82 (33.60%)	46.34	34.14		
Age (in Years)					
0-9	13 (5.32%)	46.15	46.15	0.6357	0.9298
10-18	27 (11.06%)	40.74	37.03		
19-45	115 (47.13%)	50.43	37.39		
46-65	68 (27.86%)	45.58	33.82		
>65	21 (8.60%)	33.33	33.33		
Mean	39.03	-	-		
Median (Range)	40 (1-89)	-	-		
TLC (cells/cumm)					
≤ 50000	175 (71.72%)	44	34.28	0.2498	0.2588
>50000	69 (28.27%)	52.17	42.02		
Mean	60424	-	-		
Median/Range	19990 (600-1230000)	-	-		
Platelet Count (cells/cumm)					
≤ 50000	143(58.60%)	44.05	32.86	0.4015	0.1644
>50000	101(41.39%)	49.5	41.58		
Mean	79330	-	-		
Median/Range	40000 (300-1640000)	-	-		
Hb Level (g/dL)					
≤ 8	146 (59.83%)	38.35	28.76	0.0010*	0.0012*
>8	95 (38.93%)	60	49.47		
Mean	7.65	-	-		
Median/ Range	7.55 (1-16.5)	-	-		

*Indicates statistically significant

Karyotype analysis revealed that gross karyotypic abnormalities (including aneuploidy, translocations, inversions, deletions) were detected in approximately 38.9% patients and was significantly correlated with poor survival rates at both 1 year and 5 years of time intervals (Table 2). While hyperploidy was detected in ~9% patients, hypoploidy was observed for ~ 7.8% patients. Hyperploidy was significantly correlated with poor survival at both 1 year and 5 years

of time points while hypoploidy was associated with poor OS at 1 year of time point only. Among 203 patients with normal diploidy, only 54 patients (26.3%) had no associated patient survival (Table 2).

Table 2 Karyotype status of 244 AML patients at initial diagnosis and their correlation with 1 year and 5 years of overall survival (OS)

Groups	% OS Survival		p-value	
	1-year	5- year	1-year	5- year
CN-AML (n=149) *vs.*	53.02	42.95	0.0075*	0.0086*
AML (n=95)	35.78	26.31		
46 (n=203) *vs.*	51.23	40.39	0.0038*	0.0142*
Hyperploidy (n=22)	18.18	13.63		
46 (n=203) *vs.*	51.23	40.39	0.0422*	0.0987
Hypoploidy (n=19)	26.31	21.05		
46 With aberration (n=54) *vs.*	53.02	33.33	0.3601	0.2182
CN-AML (n=149)	46.29	42.95		
*Indicates statistically significant				

Among specific chromosomal aberrations, translocation t (8;21) (q22;q22) was the most common and detected in 24 (9.8%) patients (Table 3). It was either the sole abnormality (9 patients), associated with loss of sex chromosomes (8 patients) or in combination with other cytogenetic abnormalities (7 patients). Trisomy of chromosome 8 was the most frequent sole abnormality detected in 6.5% (16) patients, other chromosomal aberrations included monosomy of chromosomes 7 or 5 and inv16 along with t (9;22) (q34;q11). None of the cytogenetic aberrations were individually found to be significantly associated with patient survival (Table 3).

Table 3 Frequency of cytogenetic abnormalities among AML patients at initial diagnosis and their correlation with 1 year and 5 years of overall survival (OS)

S. No.	Karyotypic Abnormalities	No. of Patients (%)	p-value	
			1-year	5 year
1	t (8;21)(q22;q22) sole	9 (3.68%)	ND*	ND*
	t (8;21)(q22;q22)+loss of sex chromosome	8 (3.27%)	ND*	ND*
	t (8;21)(q22;q22)+other chromosomal anomalies	7 (2.86%)	ND*	ND*
	Total t (8;21)(q22;q22)	24 (9.83%)	0.8959	0.3169
2	Trisomy 8	16 (6.55%)	ND*	ND*
3	-7/del (7q)	6 (2.45%)	ND*	ND*
4	-5/del (5q)	4 (1.63%)	ND*	ND*
5	Inversion16	6 (2.45)	ND*	ND*
6	t (9;22)(q34;q11)	3 (1.22%)	ND*	ND*
*Not determined as the number of patients was very low				

Table 4 Multivariate analysis of 244 AML patients for karyotype and other patient-specific parameters, and correlation with 1 year and 5 years of overall survival (OS)

Variable 1	Variable 2	Variable 3	Variable 4	Variable 5	p-value	
					1-year	5-year
Karyotype	Hb	-	-	-	0.002070*	0.001642*
Karyotype	Age	-	-	-	0.281535	0.147005
Karyotype	TLC	-	-	-	0.220624	0.149577
Karyotype	Platelets	-	-	-	0.261485	0.091416
Karyotype	HB	TLC	-	-	0.003906*	0.003304*
HB	TLC	-	-	-	0.002233*	0.002596*
HB	Platelets	-	-	-	0.003179*	0.001931*
HB	Age	-	-	-	0.002988*	0.002735*
HB	TLC	Platelets	-	-	0.005254*	0.003409*
HB	TLC	Platelets	Age	-	0.008855*	0.004910*
HB	TLC	Platelets	Age	Karyotype	0.010955*	0.004610*
*Indicates statistically significant						

Multivariate analysis showed that combining karyotype with Hb levels correlated significantly with both 1 year and 5 years of OS survival of AML patients (Table 4); however, significance levels were, not much different than those observed if the Hb level was considered as the sole parameter (Table 1). Further, while combining Hb level with other clinical parameters such as age, platelet count and TLC demonstrated a significant correlation with OS, the significance levels were either equivalent or lower than those observed if Hb level was considered as the sole parameter (Table 4).

DISCUSSION

Acute myeloid leukemia is a very heterogeneous disease as evidenced by an increasing spectrum of driver gene mutations that are being integrated for a better understanding of the cellular mechanisms responsible for disease initiation and progression and hold great promise for the development of more effective treatment modalities [26,31]. As the treatment regimen for AML has remained unchanged, it is important to have reliable markers to predict treatment outcomes to identify patients who may be good candidates for alternative treatment modalities like allogenic HSCT that may overcome poor prognosis or participation in clinical trials for experimental treatments like immunotherapy [32,33]. Genetic abnormalities form the remaining primary basis for AML classification and are the strongest prognostic factors [4,19-21]. Specific NPM1 and CEBPA mutations and FLT-3-internal tandem duplication are already being in clinical practice in the developed countries for predicting treatment outcomes; mutations in RUNX1, TP53 and ASXL1 are being fast-tracked for inclusion. Nevertheless, even subgrouping of AML based on genetic profiles are unable to reliably predict treatment response rates or identify patients who will develop resistance to chemotherapy or will relapse [19,20,23]. Apart from genetic profiling, prognostic factors like age, WBC count, immunophenotypic characteristics of blast cells, performance status, Myc protein expression, serum ferritin levels and leukemic stem cell marker expression [16,22,34-37].

However, none of these "prognostic factors" can reliably predict disease outcome; multiple factors need to be taken into account to improve the prediction of treatment outcomes. This is true even for genetic abnormalities where the prognostic value of an individual genetic lesion is often dependent on the presence or absence of other genetic abnormalities [6,19-21,31]. There is a general consensus that consideration of multiple independent factors can significantly improve the prediction of treatment outcomes. Along with genetic lesions, patient-specific factors such as age, performance status and comorbidities along with clinical parameters such as WBC count have been considered for construction of multicomponent models [19,21,22,26,27]. In almost all these studies, combining genetic status with clinical parameters significantly improved the prognostic reliability.

Though a high degree of prognosis is now possible with detailed genetic analysis in combination with other prognostic factors, translation of this information in clinical settings has been limited. Though there is increasing stress on detailed molecular profiling for grouping/subgrouping of AML patients with prognostic relevance to determine appropriate course of treatment [5], techniques such as real-time quantitative PCR, multi-parameter flow cytometry and next-generation sequencing are often not possible to be performed at site, and have to be referred to experienced laboratories off-site. Further, they add to the costs and opens up the perennial costs vs. benefit debate. This is of more relevance to the healthcare system of developing countries with huge populations like China and India, and also for underdeveloped countries of Africa where limited resources and technologies exist in the public healthcare system.

In light of the above, there is an urgent need to develop easy, lost-cost, prognostic tools that may help clinicians in making more rational decisions. In this pilot study, we demonstrate that apart from karyotype status, Hb level is also an independent factor influencing patient survival at 1 year and 5 years of time-points, taken either alone or in combination with karyotype or other clinical parameters such as age, TLC or platelet count. This is perhaps the first study where Hb levels have been associated with OS survival of AML patients. The measurement of Hb levels is easy and inexpensive and should be taken into account for the prognosis of AML patients. This study has a number of limitations that include:

- The small sample size is taken from a single location
- A relatively young study population with a mean age of 39 years
- Detection of gross karyotypic abnormalities only in 38.9% of the patients

The low mean age and frequency of karyotypic abnormalities may in part account for relatively high OS observed in the current study.

CONCLUSION

Though these findings need to be confirmed in a much larger patient population from multiple sites, our study suggests that all information available for AML patients should be taken into account for construction of multi-parameter models to predict prognosis of AML patients, and identify patients that are more likely to be refractory to standard treatment or suffer relapse of the disease. Ideally, all available demographic, patient-specific and disease-specific factors should be considered during the construction of multifactorial models along with genetic profiles. Even though not significant individually, these factors may significantly increase the prognostic value of the genetic profile alone. It may help in early identification of patients who are unlikely to benefit from the conventional intensive induction therapy, induct them for other therapies such as HCT or immunotherapy, or enroll them in clinical trials for experimental therapies. Identification of patients early in disease progression for alternative treatment modalities may significantly improve chances of disease remission and eventually lead to a significant reduction in AML-related mortality.

DECLARATIONS

Acknowledgments

We greatly acknowledge all the patients who kindly consented to be a part of the study. The authors also thank the whole-hearted support and encouragement from the management of Indraprastha Apollo Hospitals, and Dr. Ashok K Chauhan, Founder President, Amity Group of Institutions.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

REFERENCES

[1] Döhner, Hartmut, Daniel J. Weisdorf, and Clara D. Bloomfield. "Acute myeloid leukemia." *New England Journal of Medicine,* Vol. 373, No. 12, 2015, pp. 1136-52.

[2] Béné, M. C., et al. "Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10." *Leukemia,* Vol. 25, No. 4, 2011, pp. 567-74.

[3] Prada-Arismendy, Jeanette, Johanna C. Arroyave, and Sarah Röthlisberger. "Molecular biomarkers in acute myeloid leukemia." *Blood Reviews,* Vol. 31, No. 1, 2017, pp. 63-76.

[4] Arber, Daniel A., et al. "The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia." *Blood,* Vol. 127, No. 20, 2016, pp. 2391-405.

[5] Döhner, Hartmut, et al. "Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet." *Blood,* Vol. 115, No. 3, 2010, pp. 453-74.

[6] Döhner, Hartmut, et al. "Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel." *Blood,* Vol. 129, No. 4, 2017, pp. 424-47.

[7] Othus, Megan, et al. "Declining rates of treatment-related mortality in patients with newly diagnosed AML given 'intense' induction regimens: a report from SWOG and MD Anderson." *Leukemia,* Vol. 28, No. 2, 2014, pp. 289-92.

[8] Wang, Yuhui, et al. "Recurrent fusion genes in leukemia: an attractive target for diagnosis and treatment." *Current Genomics,* Vol. 18, No. 5, 2017, pp. 378-84.

[9] Stuart, Robert K., et al. "REVEAL-1, a phase 2 dose regimen optimization study of vosaroxin in older poor-risk patients with previously untreated acute myeloid leukemia." *British Journal of Haematology,* Vol. 168, No. 6, 2015, pp. 796-805.

[10] Lübbert, Michael, and Andrea Kuendgen. "Combining DNA methyltransferase and histone deacetylase inhibition to treat acute myeloid leukemia/myelodysplastic syndrome: achievements and challenges." *Cancer,* Vol. 121, No. 4, 2015, pp. 498-501.

[11] Shih, Alan H., et al. "Combination targeted therapy to disrupt aberrant oncogenic signaling and reverse epigenetic dysfunction in IDH2-and TET2-mutant acute myeloid leukemia." *Cancer Discovery,* Vol. 7, No. 5, 2017, pp. 494-505.

[12] Blum, William, et al. "Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia." *Journal of Clinical Oncology,* Vol. 25, No. 25, 2007, pp. 3884-91.

[13] Fenaux, Pierre, et al. "Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia." *Journal of Clinical Oncology,* Vol. 28, No. 4, 2009, pp. 562-69.

[14] Walter, Roland B. "Investigational CD33-targeted therapeutics for acute myeloid leukemia." *Expert Opinion on Investigational Drugs,* Vol. 27, No. 4, 2018, pp. 339-48.

[15] Norsworthy, Kelly J., et al. "FDA Approval Summary: Mylotarg for Treatment of Patients with Relapsed or Refractory CD33-Positive Acute Myeloid Leukemia." *Oncologist,* Vol. 23, No. 9, 2018, pp. 1103-08.

[16] Creutzig, Ursula, et al. "Acute myelogenous leukemia in adolescents and young adults." *Pediatric Blood and Cancer,* Vol. 65, No. 9, 2018, p. e27089.

[17] Keegan, Theresa HM, et al. "Comparison of cancer survival trends in the United States of adolescents and young adults with those in children and older adults." *Cancer,* Vol. 122, No. 7, 2016, pp. 1009-16.

[18] Juliusson, Gunnar, et al. "Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry." *Blood,* Vol. 113, No. 18, 2009, pp. 4179-87.

[19] Patel, Jay P., et al. "Prognostic relevance of integrated genetic profiling in acute myeloid leukemia." *New England Journal of Medicine,* Vol. 366, No. 12, 2012, pp. 1079-89.

[20] Metzeler, Klaus H., et al. "Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia." *Blood,* Vol. 128, No. 5, 2016, pp. 686-98.

[21] Papaemmanuil, Elli, et al. "Genomic classification and prognosis in acute myeloid leukemia." *New England Journal of Medicine,* Vol. 374, No. 23, 2016, pp. 2209-21.

[22] Walter, Roland B., et al. "Prediction of early death after induction therapy for newly diagnosed acute myeloid leukemia with pretreatment risk scores: a novel paradigm for treatment assignment." *Journal of Clinical Oncology,* Vol. 29, No. 33, 2011, pp. 4417-23.

[23] Walter, Roland B., et al. "Effect of genetic profiling on the prediction of therapeutic resistance and survival in adult acute myeloid leukemia." *Leukemia,* Vol. 29, No. 10, 2015, pp. 2104-07.

[24] Creutzig, Ursula, et al. "Significance of age in acute myeloid leukemia patients younger than 30 years: a common analysis of the pediatric trials AML-BFM 93/98 and the adult trials AMLCG 92/99 and AMLSG HD93/98A." *Cancer: Interdisciplinary International Journal of the American Cancer Society,* Vol. 112, No. 3, 2008, pp. 562-71.

[25] Metzeler, Klaus H., et al. "Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia." *Blood,* Vol. 128, No. 5, 2016, pp. 686-98.

[26] Malagola, Michele, et al. "A simple prognostic scoring system for newly diagnosed cytogenetically normal acute myeloid leukemia: a retrospective analysis of 530 patients." *Leukemia and Lymphoma,* Vol. 52, No. 12, 2011, pp. 2329-35.

[27] Jaffe, R. "WHO classification of tumors of hematopoietic and lymphoid tissues." *World Health Organization Classification of Tumours*, 2008, pp. 358-60.

[28] Lawce, Helen J., and Michael G. Brown. "Cytogenetics: an overview." *The AGT Cytogenetics Laboratory Manual*, 2017, pp. 25-85.

[29] International Standing Committee on Human Cytogenetic Nomenclature., Shaffer LG, Slovak ML, Campbell

LJ. ISCN 2009 : an international system for human cytogenetic nomenclature. Karger, 2009, https://www.karger.com/Book/Home/244102

[30] Bullinger, Lars, Konstanze Döhner, and Hartmut Döhner. "Genomics of acute myeloid leukemia diagnosis and pathways." *Journal of Clinical Oncology,* Vol. 35, No. 9, 2017, pp. 934-46.

[31] Ikegawa, Shuntaro, et al. "Allogeneic hematopoietic stem cell transplant overcomes poor prognosis of acute myeloid leukemia with myelodysplasia-related changes." *Leukemia and Lymphoma,* Vol. 57, No. 1, 2016, pp. 76-80.

[32] Przespolewski, Amanda, Andras Szeles, and Eunice S. Wang. "Advances in immunotherapy for acute myeloid leukemia." *Future Oncology,* Vol. 14, No. 10, 2018, pp. 963-78.

[33] Weisser, Martin, et al. "Advanced age and high initial WBC influence the outcome of inv (3) (q21q26)/t, 3; 3) (q21; q26) positive AML." *Leukemia and Lymphoma,* Vol. 48, No. 11, 2007, pp. 2145-51.

[34] Lanza, Francesco, et al. "Prognostic value of immunophenotypic characteristics of blast cells in acute myeloid leukemia." *Leukemia and Lymphoma,* Vol. 13, No. 1, 1994, pp. 81-85.

[35] Ohanian, Maro, et al. "MYC protein expression is an important prognostic factor in acute myeloid leukemia." *Leukemia and Lymphoma,* Vol. 60, No. 1, 2019, pp. 37-48.

[36] Ihlow, Jana, et al. "AML: high serum ferritin at initial diagnosis has a negative impact on long-term survival." *Leukemia and Lymphoma,* Vol. 60, No. 1, 2019, pp. 69-77.

[37] Yabushita, Tomohiro, et al. "Expression of multiple leukemic stem cell markers is associated with poor prognosis in de novo acute myeloid leukemia." *Leukemia and Lymphoma,* Vol. 59, No. 9, 2018, pp. 2144-51.