Kasr.El-Aini.J. Clin. Oncol. Nucl.Med.
Vol.2, no.3, Jul. 2006:21-24

NEMROCK

Original Article

ROLE OF FISH ANALYSIS IN THE PREDICTION OF TREATMENT OUTCOME IN ADULT PATIENTS WITH DE NOVO AML

Tamer Elnahas[1], Yasser Abdelkader[1], Hamdy Zawam[1], Yasser Hassan[2], Amina Abdelwahed[1], Mazen Elsharkawy.[1]
[1]Clinical Oncology Center (NEMROCK), Kasr EL-Aini, Faculty of Medicine, Cairo University Egypt,
[2]Clinical Pathology Department, NCI,

ABSTRACT

Patients and Methods: During the years 2001-2004, a total number of 28 patients with de novo AML were analyzed by FISH to compare the prognostic impact of certain hematological, clinical parameters and cytogenetic abnormalities on CR rates, disease free interval, and the two years OAS. The number of induction courses and the treatment outcome of dose intensification in the post remission therapy was evaluated. Fifteen AML patients received one course of induction chemotherapy, 8 patients received two courses, and five patients received three induction courses.

Results: The CR rate after the first induction course was 54% compared to 21% after the second and third induction courses (p=0.01). Patients who did not achieve CR after the first course of induction had lesser chance to achieve it after second or third courses. While stratification of patients according to hematological data as TLC, blood Hb, number of platelets in peripheral blood and clinical parameters as performance status at presentation failed to predict prognosis, FISH analysis could distinguish three groups of patients. A favorable group of cytogenetic abnormalities including five patients with t (8,21), a second group of unfavorable cytogenetic abnonormalities including 11 patients with trisomy 8 (±8) anomaly, and a third intermediate cytogenetic risk group of 10 patients having no cytogenetic abnormalities. The favorable cytogenetic group conferred significantly higher CR rate, longer disease free interval, longer overall survival, and achieved CR only after one course of induction chemotherapy in comparison with the unfavorable cytogenetic group (P<0.05). Dose intensification in the post remission therapy did not add any survival advantage to patients in this study.

Key Words: Fish analysis, AML, cytogenetic risk, treatment outcome

Correspomding Author: Tamer El-Nahas, Clinical Oncology Center (NEMROCK), Faculty of Medicine, Cairo University, Tel.:0123955039

INTRUDUCTION

Acute myeloid leukemia (AML) is a malignant bone marrow disease in which primitive cells (myeloblasts) expand, accumulate and suppress normal hematopoietic activity. AML is a rare disease, with an incidence of 1.4 cases per 100,000 in the population below 65 years old, and 13.4 per 100,000 in the population aged above 65 years, and in patients with bone marrow failure manifesting with infections, bleeding and anemias.[1-3] The pathogenesis of AML is uncertain ,but chromosomal and genetic abnormalities as well as toxic exposures are the main documented contributing factors[3-5]. Chromosome translocations result in the translation of fusion proteins, which are common pathways for leukomogenesis[4,5]. New diagnostic tools as fluorescent in situ hybridisation (FISH), and polymerase chain reaction (PCR) have improved the sensitivity of detection of chromosomal abnormalities, the ability to assess the response to treatment and detecting minimal residual disease.[6] Advances in therapies and the development of the new generation of gene based therapeutics have changed the cure rates in AML from less than 20% in 1960s to become actually between 40% to 70% for selected group.[6,7]

The recent WHO classification of AML[8] included cytogenetic risk status to morphology, cytochemistry, and immunophenotyping classification proposed by the French- American- British (FAB) group. Multiple gene rearrangements and chromosome abnormalities are found in up to two thirds of AML patients. Age and cytogenetic abnormalities were among the important prognostic factors in AML according to several studies[9]. Prognostic factors in AML patients are useful to elect the type and intensity of post remission treatment. Most progress in improving the outcome of treatment for AML has come from intensification of post-remission therapy.[10]

The present study aims at:

1. Assessing the pattern of some cytogenetic abnormalities in a number of AML Egyptian patients and their impact among other clinical factors on response to treatment and on prognosis.

2. Evaluating the effect of dose intensification in the post remission therapy.

PATIENTS AND METHODS

Twenty eight patients made the subject of this study. Their age ranged from 15-60 years old and subjected to thorough clinical, radiological and ultrasound examinations. Hemtological studies of Peripheral blood and bone marrow aspirates were performed aided by cytochemical tests and immunophenotyping by FACS analysis using fluorescent monoclonal antibodies from BD and Dakocytomation. Fluorescent in Situ Hybridisation (FISH) for cytogenetic studies was performed using probes from Vysis, for the commonest anomalies, t(8:21), t(15:17), and inv 16. For numerical abnormalities of chromosome 8 Cep 8 probe was used. The details of FISH analysis is mentioned elsewhere[4,11]. It was performed on interphase and metaphase nuclei after short term culture of peripheral blood or bone marrow aspirates. After initial confirmation of diagnosis, the stabilization of the patients general condition was attempted by necessary blood or component transfusions to reach a blood hemoglobin level of 8gm/dl, and platelet count of 3(10)^9/l. Fever was controlled by IV antibiotics, for at least 48 hours before the initiation of treatment. The induction therapies administered to patients are summarized in table 1. Patients achieving complete remission were randomly assigned to two consolidation protocols a standard and an intensified one, as seen in table 1, patients were also assessed for toxicity after each cycle of chemotherapy. Daily complete blood counts were done, blood and other body fluid cultures were done in case of fever or neutropenia and specific antibiotic therapy was immediately applied. Myelogramme was performed following completion of consolidation and maintenance therapy, and then every three months during the first year and then every 6 months during the second year. Patients were also assessed for toxicity after each cycle of chemotherapy according to WHO criteria[12,13]. Standard statistical methods were applied for data analysis. Fisher's exact test was used to compare percentages. Kaplan and Meyer method was used for the estimation of over all survival (OAS), and disease free interval (DFS). The log Rank test was used to compare between survival curves.

Table 1: Treatment Protocoles of The Studies AML cases.

Patients	Induction	Consolidation		Maintenace
	7-3 Protocole	Arm A	Arm B	
All AML cases Except M3	Doxorubicin 30 mg/m² d1-d3 IV infusion over 15 min Ara c 100 mg/m² IV continuous infusion over 24 hrs d1-d7 3rd genration antiserotonin antiemetic IV 30 min. before start of treatment	Ara C 3 gm/m² every 12 hrs d1,3,5 (4 Cycles at 3 wks interval	Ara C 2 gm/m² every 12 hrs d 1,3,5 (3 Cycles at 3 wksinterval)	
M3 cases	ATRA 45 mg/ m² /d in two divided doses for 45-90 days			Intemittent ATRA 45 mg/m² Every 3 months

RESULTS

This study included 28 patients presented to NEMROCK during the period 2001-2004, diagnosed as de novo AML according to morphological cytochemical and immunophenotyping criteria of blood and bone marrow. Their age ranged from 15-60 years with equal number of males and females. Table 2 shows the performance status of these patients at presentation. Fever and anemia were the commonest presenting symptoms followed by weight loss and bleeding tendencies. Nine patients had lymphadenopathies, 8 patients suffered from hepatomegaly and 7 patients had splenomegaly. Leucocytic counts varied from severe leucopenia to high leukemic counts. All the patients presented with grade 1 to grade four thrombocytopenia.

Table 2: Patients characteristics at presentation.

Study group	28 patients
Diagnosis	De novo AML
♂: ♀	1:1
Age range	15-60 yrs.
Performance status	
ECOG Scale I	10 patients
ECOG Scale II,III	18 patients

Classification of the 28 patients according to FAB criteria showed that M1 and M2 are the most common classes, (Table 3). The results from cytogenetic study by FISH analysis is demonstrated on table 4. The t (8,21) alone was found in five patients, the numerical abnormality trisomy 8 (+8) was found in 11 patients as sole anomaly, inv 16 was found in one patient and t (15,17) in one patient. Ten patients had no detectable cytogenetic abnormalities by FISH analysis.

Table 3: Induction treatment results according To FAB class.

FAB Class	DFS (Mo)	CR after one Induction	CR after 2-3 Inductions
M0 (1)	19	1	0
M1 (6)	14.7	4	1
M2 (9)	15	5	3
M3 (2)	2	0	1
M4 (3)	6	2	0
M5 (6)	6.7	2	1
M6 (1)	6	1	0
Total 28 P=0.01		15(54%)	6(21%)

Table 4: Cytogentic abnormalities in the different FAB classes.

FAB Class	Cytogenetic abn
M0 (1)	1 t(8;21)
M1 (6)	2 t(8;21),4(+8)
M2 (9)	2 t(8;21),7(+8)
M3 (2)	1 t(15;17)
M4 (3)	1 (Inv 16)
M5 (6	0
M6 (1)	0
Total 28	18(64%)

The different clinical and hematological parameters at presentation as performance status, blood hemoglobin, leuco cytic or platelet counts did not show any impact on response to treatment in terms of OAS or DFS, figures 1-4. The CR rate after the first induction course was 54% compared to 21% in patients receiving two or three induction courses in all FAB classes p=0.01 (Table 2). The relationship between the number of induction courses and treatment outcome in terms of OAS is shown on figure 5. A significant increase in OAS in patients achieving CR after one induction course compared to patients receiving two or more induction courses, P=0.01. The relationship between the type of cytogenetic abnormality and the response to the first induction chemotherapy course is shown on table 5. A significant survival difference was found between patients with t(8;21) compared to those with trisomy 8 (+8) (Figure 6). The OAS at two years in patients achieving CR after one induction course was 47% whereas for the whole group was 39%. Patients receiving the intensified consolidation regimen A did not show a significant difference in DFS or OAS from

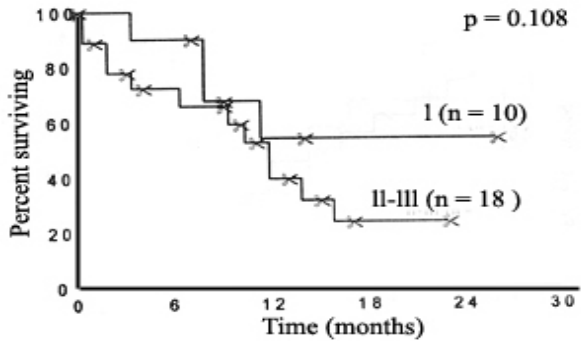

Fig. 1: Overall survival stratified according to performance status.

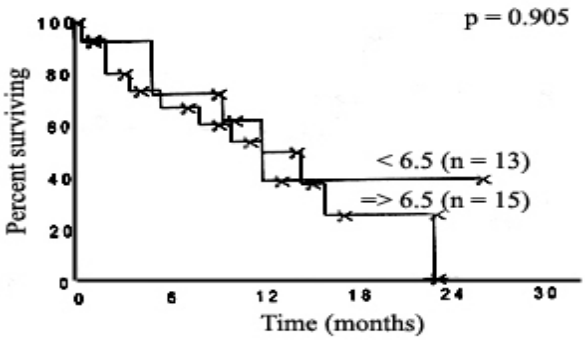

Fig. 2: Overall survival stratified according to hemoglobin level at presentation.

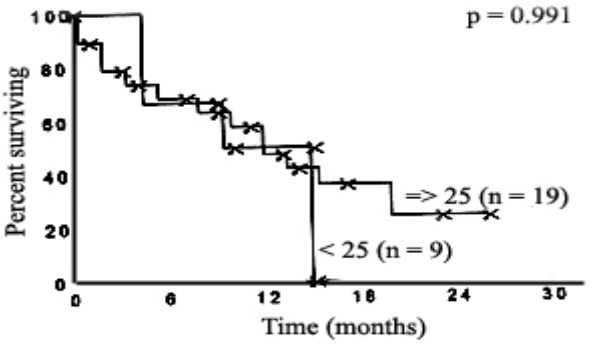

Fig. 3: Overall survival stratified according to platelet count at presentation.

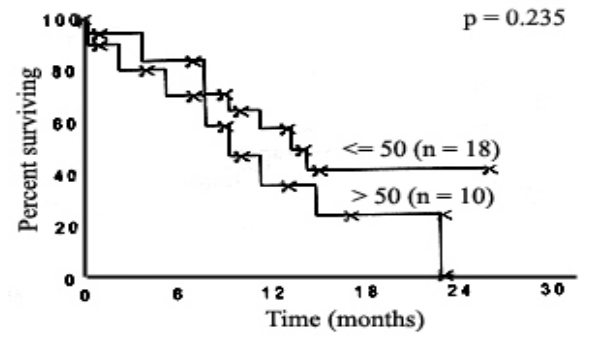

Fig. 4: Overall survival stratified according t total leucocytic count at presentation.

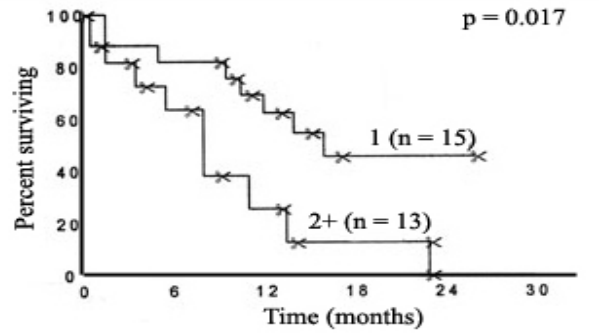

Fig. 5: Overall survival stratified according to number of inductions received.

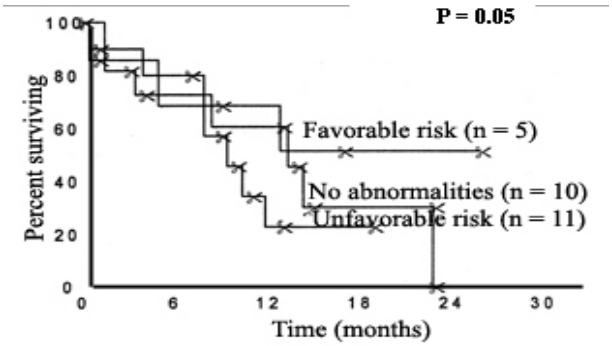

Fig. 6: Overall survival stratified according to cytogenetic risk groups.

patients of arm B receiving the standard consolidation regimen. (Figure 7)

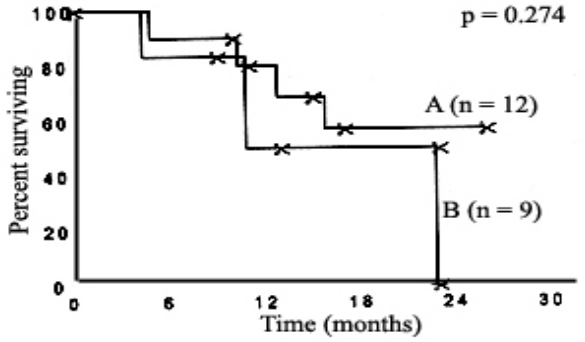

Fig. 7: Overall survival stratified according to consolidation regimen received.

Table 5: Relationship between cytogenetic abnormality and response to induction therapy.

Abnormality		CR after 1 Induction	OAS (M0.)
t(8;21)	(5)	5(100%)*	22
No abnormality	(10)	6(60%	14.5
(+8)	(11)	4(36%)*	8.3
Inv. 16	(1)	0	7
t(15;17)	(1)	0	7

*p = 0.05

DISCUSSION

The pathogenesis of AML has been better understood by the discovery of certain contributing factors, mainly chromosomal abnormalities and genetic mutations, Chromosomal translocations result in translation of fusion proteins triggering signal transduction dysregulations[9]. The identification of these cytogenetic aberrations contributed to better tailoring of the treatment policy of the patients and helped the prediction of treatment outcome.[14]

The present study of the 28 Egyptian patients with AML revealed that 18 of them (64%) were found to harbour either structural or numerical chromosomal abnormalities. The majority of these patients belonged to the FAB classes M1 and M2. Trisomy of chromosome 8 (±8) was found in 39% of them, followed by t(8;21) in about 18% (five patients) whereas 36% (10 patients) did not show any chromosomal abnormality. This pattern is different from that reported on a larger group of patients in the SWOG – ECOG study regarding mainly the frequency of the most aggressive abnormality trisomy 8 (±8) which was found to only represent 9% of the cases, a finding that may explain the modest treatment outcome in patients of the present study. The British MRC- AML 10 trial reported an OAS at five years of 44% with a significant difference between the cytogenetic risk groups[9,13]. In the present study the median OAS was[13] months with an OAS rate at two years of 30% for the whole studied group. However, patients with +8 had a survival rate at two years of only 20%, significantly lower than that of patients with t(8,21), being 50% (p=0.03). This finding adds proof to the bad prognostic impact of ±8 abnormality, and confirming the SWOG-ECOG study and the British MRC AML 10 trial correlating the cytogenetic risk status and OAS8[13]. The CR rate in our study was 60% compared to 85% reported by the MRC AML 10 trial whereas the treatment related mortality was 8% versus 11% in our group.

Complete remission was achieved after one induction course in patients[15], five patients needed two induction courses. Seven of the remaining eight patients could not get remission even after three induction courses which may be related to the prevalence of the bad cytogenetic risk status +8 or possibly to drug resistance[16]. When the CR rate is analysed in light of cytogenetic data, we found that one induction course achieved CR in 100% of patients with t (8;21), compared to CR rate of 60% in patients with no cytogenetic abnormality and 38% in patients with +8, (p=0.05), a finding consistent with other findings categorizing trisomy 8 abnormality as a bad cytogenetic risk , t(8;21) as a good one and absence of chromosomal abnormality as an intermediate cytogenetic risk status[16]. The attempt of improving the treatment outcome of the bad cytogenetic risk patients by immediate post remission intensification chemotherapy did not add to the OAS or to the DFS. On the contrary it has led to higher grades of hematological toxicities, which proves again the value of bone marrow transplantation from a related matched donor as an elective post remission therapeutic approach[17,18]. On the other hand the identification of the molecular defects triggering aberrant signaling pathways in AML stimulated the development of new generation of targeted therapies that are actually under intensive clinical trials and may contribute to an important modification of the natural history of AML.[19]

REFERENCES

1. Brunning RD, McKenna RW. Acute leukemias. In: Brunning RD, McKenna RW, editors. Tumors of the bone marrow.Washington: Armed forces institute of pathology; 1994 p. 19-142.

2. Greer JP, Baer MR, Kinney MC. Acute myelogenous leukemia in children. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, editors. Wintrobe's clinical hematology: Lippincott Williams & Wilkins; 1998 p. 2272-319.

3. Ross JA. Epidemiology and hereditary aspects of acute leukemia. In: Wiernik PH, Goldman JM, Dutcher JP, Kyle RA, editors. Neoplastic diseases of the blood. 4th ed. UK: Cambridge university press; 2003 p. 164-75.

4. Dewald GW, Stupca P. 154 Chromosome Anomalies in Hematologic Malignancies. Leuk Res 2000 Jun;24(6):487-9.

5. Dimartino JF, Cleary ML. Mll rearrangements in haematological malignancies: Lessons from clinical and biological studies. Br J Haematol 1999 Sep;106(3):614-26.

6. Willman CL. Molecular evaluation of acute myeloid leukemias. Semin Hematol 1999 Oct;36(4):390-400.

7. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: A Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 2000 Dec 15;96(13):4075-83.

8. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller Hermelink HK, Vardiman J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997 J Clin Oncol 1999 Dec;17(12):3835-49.

9. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: Analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998 Oct 1;92(7):2322-33.

10. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med 1994 Oct 6;331(14):896-903.

11. Paietta E, Papenhausen P. Cytogenetic alterations and related molecular consequences in adult leukaemia. In: Steele GD, Phillips TL, Chabner BA, Gansler TS, editors. American cancer society atlas of clinical oncology: adult leukaemias.Canada: B.C. Decker; 2001 p. 161-90.

12. Elonen E, Almqvist A, Hanninen A, Jansson SE, Jarventie G, Koistinen P, et al. Comparison between four and eight cycles of intensive chemotherapy in adult acute myeloid leukemia: A randomized trial of the Finnish Leukemia Group. Leukemia 1998 Jul;12(7):1041-8.

13. Rees JK, Gray RG, Wheatley K. Dose intensification in acute myeloid leukaemia: Greater effectiveness at lower cost. Principal report of the Medical Research Council's AML9 study. MRC Leukaemia in Adults Working Party. Br J Haematol 1996 Jul;94(1):89-98.

14. Raimondi SC, Chang MN, Ravindranath Y, Behm FG, Gresik MV, Steuber CP, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: Clinical characteristics and treatment outcome in a cooperative pediatric oncology group study-POG 8821. Blood 1999 Dec 1;94(11):3707-16.

15. Burnett AK. Acute myeloid leukemia: Treatment of adults under 60 years. Rev Clin Exp Hematol 2002 Mar;6(1):26-45; discussion 86-7.

16. Leith CP, Kopecky KJ, Godwin J, McConnell T, Slovak ML, Chen IM, et al. Acute myeloid leukemia in the elderly: Assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 1997 May 1;89(9):3323-9.

17. Burnett AK, Wheatley K, Goldstone AH, Stevens RF, Hann IM, Rees JH, et al. The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: Results of the UK MRC AML 10 trial. Br J Haematol 2002 Aug;118(2):385-400.

18. Linker CA, Damon LE, Ries CA, Navarro WA, Case D, Wolf JL. Autologous stem cell transplantation for advanced acute myeloid leukemia. Bone Marrow Transplant 2002 Feb;29(4):297-301.

19. Giles FJ. New drugs in acute myeloid leukemia. Curr Oncol Rep 2002 Sep;4(5):369-74.