Clinical Applications of PML-RAR α Transcript in Acute Promyelocytic Leukemic Adult Egyptians

Samir Abdulla[1], Tawfik Eladl[1], Ashraf Talaat[1], Nabil Khattab[1], Abdulshafy Tabl[1], Mohamed Samra[2] and Yasser Elnahas[3]

[1]Internal Medicine Department, Faculty of Medicine, Benha University
[2]Medical Oncology&Hematology and Clinical Pathology Departments, National Cancer Institute, Cairo University
drsamirelsharkawy@yahoo.com

Abstract: Background: Acute Myeloid Leukemia (AML) is a malignant clonal disorder of immature hematopoietic cells. Leukemic blasts may express abilities for maturation to a variable degree, which leads to morphologic heterogeneity. AIM: This study aimed at detection of PML-RAR by real-time quantitative polymerase chain reaction (RQ-PCR) for monitoring minimal residual disease (MRD) in patients with acute promyelocytic leukemia (APL),and to study the clinical application of RQ-PCR of APL for detection of risk of relapse in different phases of treatment, comparing these data with those yielded by conventional qualitative reverse transcriptase-PCR.PATIENTS AND METHODS: Twenty one consecutive patients diagnosed as acute myeloid leukemia (M3) were included in this prospective study. Patients with cardiac and respiratory diseases were excluded. All patients were subjected to the following: full history taking, complete clinical examination, some laboratory tests(complete blood picture &serum creatinine &serum alanine aminotransferase and serum bilirubin).Bone marrow asprite (BMA)for morphology and imunophenotyping (IPT) and cytogenetic studies as well as Real –time Qualitative PCR (RT-PCR)for detection of PML-RAR α gene in BMA at diagnosis and after consolidation were done.Quantitative PCR (RQ-PCR) in BMA sample after induction phase and consolidation phase to detect normalized copy number (NCN) of PML-RAR α was also done. Samples were taken after informed consent, bone marrow (BM) samples were collected from APL patients into tubes containing EDTA anticoagulant before treatment for RT-PCR, after the induction therapy and after consolidation therapy for RQ-PCR. All patients received AML M3 protocol in the form of Induction phase: ATRA: 45mg\m2 divided into two doses orally till complete remission or maximally for 90 days; and Dounroubicin or doxorubicin: 60 mg/m2 I.V. for 3days (1 course).Also, consolidation phase protocol was given in two courses of dounrobicin or doxorubicin: 60 mg/m2 IV for 3 days every month for two months. According to RQ-PCR results after consolidation phase; patients were divided into two groups, group (A): NCN OF PML-RAR α ≤1(these patients will be kept on follow up without treatment with ATRA),and group (B): NCN of PML –RAR α >1 or leukocyte count at diagnosis >10000/cmm(These patients received maintenance treatment in the form of oral mercaptopurine, methotrexate and intermittent ATRA for up to 2 years). During this period; patients were kept on follow up for detection of relapse or remission which is defined as: Hematological remission in the form of normalization of peripheral blood and BMA<5%blasts and no promyelocyte in peripheral blood and BMA<5%blasts.Hematological relapse was considered in the form of reappearance of >5%blasts in BMA or promyelocyte. RESULTS: There was no statistical difference between the two groups as regarding age and gender, Hb levels, Platelets count, TLC count, PB Promyelocytes(%), BM promyelocyte and serum fibrinogen level. There was statistical difference between the two groups as regarding OS; in group A was (13+17.8) and group B was (7.39+ 13)(p.>0.05).There was statistical difference between the two groups as regarding DFR, in group A it was (12+24.00) and group B was (8.014+ 2) .There was no statistical correlation between OS & DFR and hemoglobin levels, platelets count, TLC,PB promyelocyte mean serum fibrinogen level , NCN 1 and BM promyelocyte. There was statistical correlation between OS& DFR and NCN2 (post consolidation)(p<0.05). There was no statistical correlation between NCN1 (post induction) and NCN2 (post consolidation) and hemoglobin level, TLC count, platelets count or PB promyelocyte. There was no statistical correlation between NCN1 and NCN2, mean serum fibrinogen and BM promyelocyte (p>0.05).CONCLUSIONS:These data suggested that RT-PCR could be used as a complementary assay for the RQ-PCR approach, especially within the subgroup with 1–10 NCN.Furthermore, it is important to note that the relatively high specifity of RT-PCR assay is not reason enough to substitute a highly sensitive, standarized and high through-put technology such as RQ-PCR. Recommendations: We recommend other study on a larger scale to study PML-RAR α transcription for risk stratification of relapse in acute promyelocytic leukemic Egyptians.

[Samir Abdulla ,Tawfik Eladl, Ashraf Talaat,Nabil Khattab, Abdulshafy Tabl, Mohamed Samra and Yasser Elnahas. **Clinical Applications of PML-RAR α Transcript in Acute Promyelocytic Leukemic Adult Egyptians.** *J Am Sci* 2013;9(2):247-255]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 34

Key words: PML-RAR α, Promyelocytic Leukemia, PCR.

Introduction

Acute promyelocytic leukemia (APL) represents 10 to 15 percent of the cases of acute myeloid leukemia in adults. This disease is characterized by a specific cytogenetic abnormality i.e. balanced reciprocal translocation between chromosomes 15 and 17, that disrupt the retinoic acid receptor α gene(RAR-α) on chromosome 17 and the promyelocytic leukemia gene (*PML*), which encodes a transcription factor and is located on chromosome 15.[1]

The resulting fusion gene, *PML–RAR-α*, encodes a chimeric protein that causes an arrest of maturation at the promyelocyte stage of myeloid cell development.[2]

Patients with APL represent a marked hemorrhagic diathesis caused by disseminated intravascular coagulation (DIC) and excessive primary fibrinolysis. Introduction of all-trans retionoic acid (ATRA) has been the major breakthrough in the treatment of APLsince 1990s , doubled the survival rate expected with chemotherapy and the complete remission rate was raised up to 90% to 95% and 5-year disease free survival (DFS) to 74% .[3]

ATRA is currently recognized as the molecular targeted therapy directed at the chimeric protein generated by the specific chromosomal translocation in APL.[4]

Also, the use of arsenic trioxide (ATO) since early 1990s further improved the clinical outcome of refractory or relapsed as well as newly diagnosed APL. Because of the excellent response with ATRA plus chemotherapy, stem cell transplantation generally is not indicated in the first CR but might be considered for the high-risk patients.[5]

Currently, patients with APL should be treated with ATRA, and anthracycline for induction, followed by chemotherapy consolidation. The role of ATRA during consolidation is not established. Maintenance therapy should include oral mercaptopurine, methotrexate and intermittent ATRA for up to 2 years. However, it is not clear whether maintenance chemotherapy actually prevents relapse in APL patients treated with ATRA and chemotherapy, especially after they have become negative for the PML-RAR α transcript at the end of intensive consolidation chemotherapy. If short-term therapy without maintenance shows DFS rates identical to those for long-term therapy with maintenance, it would be beneficial for patients' quality of life as well as for medical costs.[5]

The specific PML-RARα transcripts permit not only a precise diagnosis but also provide markers for the identification of residual or recurrent disease.[6,7]

It has become evident that PML-RARα-positive by qualitative nested reverse transcription-polymerase chain reaction (RT-PCR) after consolidation or reappearance of a positive PCR during follow up are associated with a subsequent overt hematological relapse. However, a negative result does not guarantee persistence of remission.[7,8]

A quantification of the leukemia burden by real-time quantitative RT-PCR (RQ-PCR) at diagnosis followed by a close monitoring after each treatment course and during follow up might improve the comparability of the different treatment approaches in APL and might give information on the individual prognosis.[9,10]

Aim of the Work

This study aimed at:Detection of *PML-RAR* by real-time quantitative polymerase chain reaction (RQ-PCR) for monitoring minimal residual disease (MRD) in patients with acute promyelocytic leukemia (APL) and to study the clinical application of RQ-PCR of APL for detection of risk of relapse in different phases of treatment.

2. Patients &Methods

Twenty one consecutive patients diagnosed as acute myeloid leukemia (M3) were included in this study.Their ages were ≥18 years with morphological and immunophenotypic diagnosis of AML M3. Patients had positive PML-RAR α by qualitative PCR (RT-PCR) at time of diagnosis .Patients with cardiac and respiratory diseases were excluded.All patients were subjected to the following: complete history taking,full clinical examination, complete blood picture, serum creatinine,ALT and serum bilirubin. Bone marrow asprite (BMA) for morphology, imunophenotyping (IPT) and cytogenetic studies was done. Also, Real –time Qualitative PCR (RT-PCR) was done (for detection of PML-RAR α gene in BMA at diagnosis and after consolidation therapy) and quantitative PCR (RQ-PCR) in BMA sample(after induction phase and consolidation phase) was done to detect normalized copy number (NCN) of PML-RARα.

Sample:After informed consent, bone marrow (BM) samples were collected from APL patients into tubes containing EDTA anticoagulant before treatment for RT-PCR , after the induction therapy and after consolidation therapy for RQ-PCR.[11]

All patients received AML M3 protocol in the form of : A-Induction phase:

1-ATRA: 45mg\m2 divided into two doses orally till complete remission or maximally for 90 days.2-Dounroubicin or doxorubicin: 60 mg/m2 I.V. for 3days (1 course).B-Consolidation phase: Two courses of dounrobicin or doxorubicin : 60 mg/m2 IV for 3 days every month for two months. According to RQ-

PCR results after consolidation phase ;patients were divided into two groups :Group (A): NCN OF PML-RAR α ≤1.These patients were kept on follow up without treatment with ATRA.Group (B): NCN of PML –RAR α >1 or leukocyte count at diagnosis >10000/cmm.These patients received maintenance treatment in the form of oral mercaptopurine, methotrexate and intermittent ATRA for up to 2 years. During this period: Patients were kept on follow up for detection of relapse or remission which is defined as:Hematological remission: Normalization of peripheral blood and BMA<5%blasts and no promyelocyte in peripheral blood and BMA<5% blasts. Hematological relapse was considered if reappearance of >5%blasts in BMA or promyelocytes happened.

Statistical analysis

All tests were carried out with the SPSS 12.0 program (SPSS, USA). For univariate analyses, χ^2 and Fisher's exact test were performed to evaluate factors associated with relapse. Relapse-free survival (RFS) for analysis after consolidation therapy was defined as the time between the achievement of complete remission and relapse or last follow-up. OS was defined as the time from achievement of complete remission to death or last follow-up.[12]

3. Results

The present study included 21 patients diagnosed with acute myeloid leukemia M3.They were divided according post consolidation NCN (NCN2) into two groups:Group (A): NCN2 ≥1 *and Group (B): NCN2 >1*.

There was no statistical significant difference between the two groups as regarding gender (Table1), Age (Table 2); Hb levels(g/dl), platelet count ((x10^3/ccm), TLC(x10^3/ccm) and PB promyelocyte (%) (Table3). BM promyelocyte (Table4 & Figure1), serum fibrinogen levels(Table5).There was statistical significant difference between the two groups as regarding OS, in group A it was (13±17.8) and in group B it was (7.39±13)(Table 6 & Figure 2).

There was statistical significant difference between the two groups as regarding DFR: in group A it was (122±4.00) and in group B was (8.014±2). There was no statistical significant correlation between OS & DFR and hemoglobin levels or platelets count (Table 8).There was statistical significant correlation between OS& DFR and TLC (Table 7). There was no statistical significant correlation between OS& DFR and PB promyelocyte (%) (Table 7 & Figs. 3a and b). There was no statistical significant correlation between OS& DFR and BM promyelocyte (Table 9). There was no statistical significant correlation between OS& DFR and mean serum fibrinogen level (Table10).

Post induction: there was no statistical significant correlation between OS & DFR and NCN 1(Table 11); and postconsolidation, there was statistical significant correlation between OS& DFR and NCN2 (Table 12& Figs. 4a and 4b). There was no statistical significant correlation between NCN1 (post induction) and NCN2 (post consolidation) and hemoglobin levels, TLC count, platelets count or PB promyelocytes (Table13). There was no statistical significant correlation between NCN1 and NCN2 and BM promyelocytes (Table 14). There was no statistical significant correlation between NCN1 and NCN2 and serum fibrinogen (Table 15).

Table 1**:** Statistical comparison between group (A) and group (B) as regarding gender:

	Group A (n=14)		Group B (n=7)		Total		*X2*	*p*
	No.	%	No.	%	No.	%		
Female	7	50.0%	4	57.1%	11	52.4%	0.1	>0.05
Male	7	50.0%	3	42.9%	10	47.6%		
Total	14	100.0%	7	100.0%	21	100.0%		

Table 2: Statistical comparison between group (A) and group (B) as regarding Age(years):

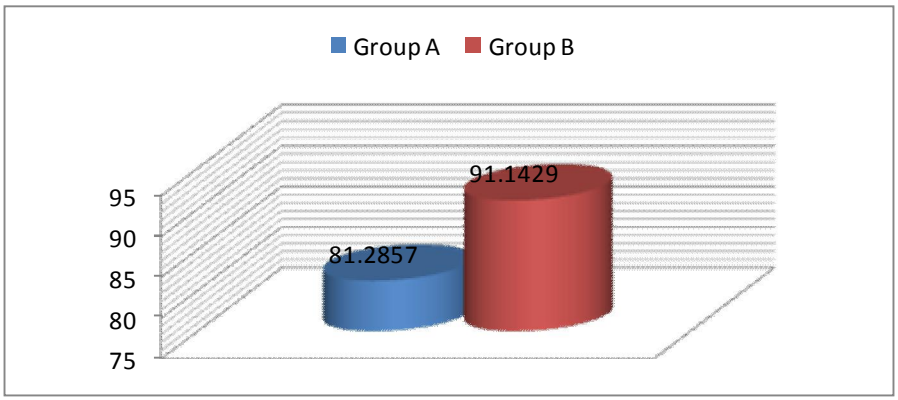
	Group A (n=14)		Group B (n=7)		Test of significance	*P*
	Mean	±SD	Mean	±SD		
Age	34.0714	8.73071	28.4286	8.14160	1.5	>0.05

Table3: Statistical comparison between group (A) and group (B) as regarding CBC:

	Group A (n=14)		Group B (n=7)		Test of significance	*p*
	Mean	±SD	mean	±SD		
Hb (g/dl)	7.6286	1.74991	9.2286	2.28890	1.8	>0.05
Platelets((x10^3ccm).	116.7141	77.672	73.714	63.27	1.3	>0.05
TLC(ccm)	32821.4286	27288.15405	40542.8571	37618.34026	0.5	>0.05
PB promyelocyte (%)	66.8571	17.29765	72.7143	18.49067	0.7	>0.05

Table 4: Statistical comparison between group (A) and group (B)as regarding BM promyelocyte%:

	Group A (n=14)		Group B (n=7)		Test of significance	*p*
	mean	±SD	mean	±SD		
BM promyelocyte	81.2857	12.30027	91.1429	7.35818	0.3	>0.05



(Figure 1)

Table 5: Statistical comparison between group (A) and group (B) as regarding mean serum fibrinogen(mg%):

	Group A (n=14)		Group B (n=7)		Test of significance	*p*
	Mean	±SD	mean	±SD		
S.Fibrinogen	224.5000	69.81046	258.5714	88.58679	0.9	>0.05

Table 6: Statistical comparison between group (A) and group (B) as regarding OS:

	Group A (n=14)		Group B (n=7)		Test of significance	*p*
	Mean	±SD	Mean	±SD		
OS(months)	13	17.8462	7.39196	13	3.002	<0.05

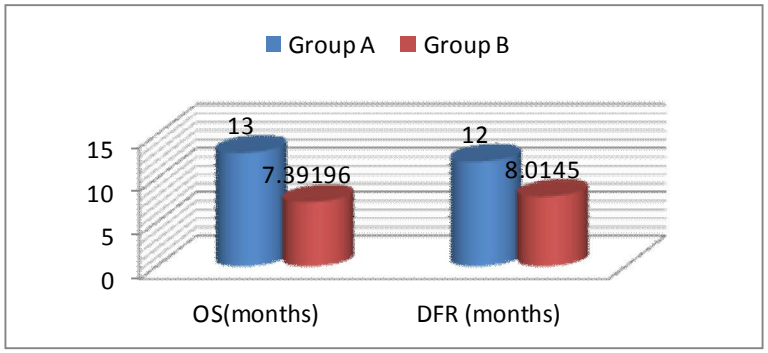


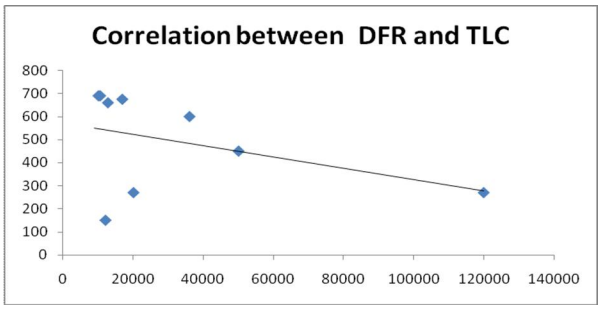
Figure 2: Statistical comparison between group (A) and group (B) as regarding OS & DFR.

Table 7: Correlation between OS & DFR and TLC (/ccm) and PB promyelocyte (%):

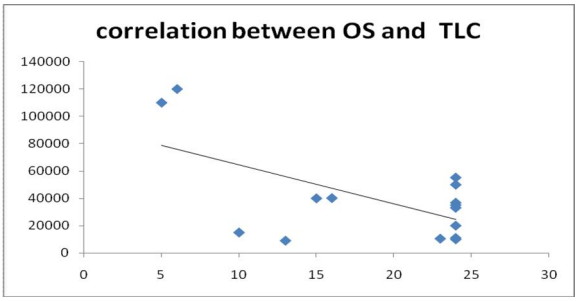
	OS		DFR	
	r	p	r	*p*
TLC	-0.552	<0.05	-0.588	<0.05
PB promylocyte	0.2790	>0.05	-.019	>0.05

Table 8:Correlation between OS & DFR and blood hemoglobin level & platelets counts:

	OS		DFR	
	r	*p*	r	*p*
Hb (gm/dl)	0.117	>0.05	-0.335	>0.05
Platelets ($x10^3$ccm)	-0.080	>0.05	-0.440	>0.05



(Figure3a)



(Figure 3b)

Table 9:Correlation between OS & DFR and BM promyelocyte (%):

	OS		DFR	
	r	*p*	r	*p*
BM promyelocyte	-0.242	>0.05	0.035	>0.05

Table10:Correlation between OS & DFR and mean serum fibrinogen levels(mg%):

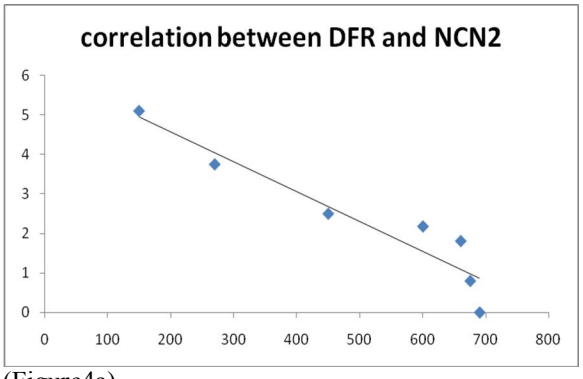
	OS		DFR	
	r	*p*	r	*P*
S.Fibrinogen	-0.067	>0.05	0.088	>0.05

Table11:Correlation between OS & DFR(months) and NCN 1(post induction):

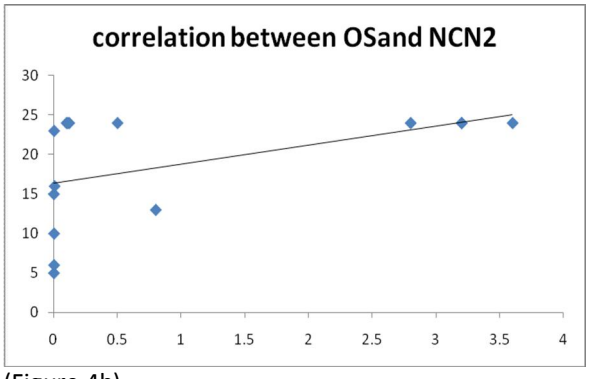
.	NCN1	
	r	*p*
DFR	0.421	>0.05
OS	0.048	>0.05

Table 12:Correlation between OS& DFR(months) and NCN 2 (post consolidation):

	NCN2	
	r	*p*
DFR	-0.94	<0.001
OS	0.62	<0.05



(Figure4a)



(Figure 4b)

Table13: Correlation between NCN1 (post induction) and NCN2 (post consolidation) and CBC:

	NCN1		NCN2	
	r	*p*	r	*p*
Hb(gm%)	0.286	>0.05	0.328	>0.05
Platelets(x10^3ccm).	-0.250	>0.05	-0.265	>0.05
TLC(ccm)	0.208	>0.05	0.172	>0.05
PB promylocyte(%)	0.220	>0.05	0.193	>0.05

Table14:Correlation between NCN1 and NCN2 and BM promyelocyte (%):

	NCN1		NCN2	
	r	*p*	r	*p*
BM promylocyte	0.338	>0.05	0.353	>0.05

Table15:Correlation between NCN1 and NCN2 and mean serum fibrinogen(mg%):

	NCN1		NCN2	
	r	*p*	r	*p*
S.Fibrinogen	0.129	>0.05	0.108	>0.05

4. Discussion

Combined treatment with anthracycline- based chemotherapy and all trans retinoic acid (ATRA) are highly successful in acute promyelocytic leukemia (APL), providing long-lasting remissions and probable cures in up to 70% of newly diagnosed patients[6,15,17].Nevertheless, the persistence of resistant clones causing relapse and low survival still represents a problem in 15–25% of patients[18,19].

Currently, detection of PML/RAR α transcripts by molecular techniques constitutes an important tool for monitoring MRD and predicting evolution in APL patients[20,21].

Conventional qualitative reverse transcriptase polymerase chain reaction (RT-PCR) has been widely used for genetic diagnosis and therapeutic monitoring of APL. Several reports have shown that RT-PCR positivity after consolidation treatment predicts hematologic relapse, whereas persistent RT-PCR negativity test is associated with long-term survival and a low relapse rate[8,22].

Using conventional reverse transcription-polymerase chain reaction (RT-PCR) assays, it was evident that PCR positivity after consolidation completion is a strong predictor of subsequent hematologic relapse[23].

Recently, quantification of the PML/RARα copy number based on Real-time PCR approaches (RQ-PCR) has become a new alternative for monitoring disease outcome. Although this approach suffers from some of the same problems as conventional RT-PCR, it has several advantages such as being highly sensitive, facilitating assessment of kinetics and being highly reproducible. Although several protocols have been developed for quantitative monitoring in APL[24-28]. In our study, no correlation between DFR &OS and mean hemoglobin level and mean platelets count (p>0.05); which is in agreement with Seok et al., and Carlos et al.,[29,30] who stated that Hb levels and platelets counts had no prognostic impact on relapse risk in patients with acute promyelocytic leukemia.

In this study, there was a significant correlation between DFR &OS and mean total leukocytes count (TLC) (p<0.05) i.e the greater number of TLC at diagnosis was inversely proportional to OS & DFR, which is in agreement with Carlos et al.,[30] who found that WBC count higher than 10000/µL (p=0.021) at diagnosis were the only parameter associated with a shorter DFR and OS[30].

In the present study, there was no correlation between DFR &OS and peripheral blood promyelocytes or bone marrow promyelocyte, this is in agreement with studies that stated parameters such as the number of blast cells in bone marrow or peripheral blood, had no prognostic impact on relapse risk or OS[30,31].

In this study ,there was no correlation between NCN after induction (NCN1) & NCN after consolidation(NCN2) and mean hemoglobin level or platelets count or peripheral blood promyelocyte ,bone marrow promyelocyte or mean serum fibrinogen level,which is in agreement with ZHU et al.,[32] who stated that there was no association of PML-RARα transcript type with sex, hemoglobin, platelet count, the percentage of promyelocytes or bone marrow or signs of clinically diagnosed coagulation/bleeding disorders.

In our study, there was no correlation between NCN after induction(NCN1) DFR or OS i.e NCN 1 has no impact on OS or DFR, which is in agreement with studies those obtained by the GIMEMA2 and PETHEMA5 groups [33]who observed no influence of PCR positivity after induction on treatment outcome or OS .Also, it is in agreement with with many authors[15,21,34,35] who stated that there is no correlation was found between a positive test immediately after induction therapy and outcome i.e actually, no significant differences in PML-RARα NCN values post-induction were observed between relapsed patients and those who remained in continuous complete remission[36].

In this study, there is correlation between NCN after consolidation (NCN2) DFR or OS i.e the greater the value of NCN 2 more than 1 was associated with shorter OS &DFR and the less value of NCN 2 less than 1 was associated with more favorable outcome. This is in agreement with many authors[6,27,37] who stated

that regarding post-consolidation test of PCR, there was correlation between positive RQ-PCR assays and a high risk of relapse. However; in their series, as well as in other studies, the low number of positive cases detected at the end of consolidation limits the utility of this parameter[38].

Also ,Cassinat et al.,[10] suggested that the MRD level after first consolidation may reflect the presence of resistant leukemic clones and, thus, that the magnitude of MRD reduction at this stage is related to clinical outcome .

In this study, there was statistical difference between the two groups as regarding OS in group A and group B. In this study also, there was statistical difference between the two groups as regarding DFR in group A and group B i.e 14 % relapsed in group(A) but 71 % patients were relapsed in group (B).This is in agreement with Miguel *et al.* [30] who found two groups with different probabilities of DFR and OS in patients with AML 3 who could be established based on the PML/RAR α NCN: <1 NCN and 1–10 NCN. And the two groups had 5-year DFR probabilities of 100%, 67% respectively.

Also, this is in agreement with other studies[10,39] who stated that the 5-year probabilities of relapse and disease-free survival were 17.3±5.4% and 81.5±5.4%, respectively in group(1) which depend on NCN post consolidation ≤1 and group (2) which depend on NCN post consolidation > 1, this is translated into a better outcome in terms of the probabilities of relapse (85.7±13.2% versus 7.3±4.1%, p<0.001) and disease-free survival (14.3±13.2% versus 91.2±4.3%, p<0.001).

Also, this is in agreement with one study[40], who stated that patients with <1 NCN had a very favorable DFR especially when the test was performed during follow-up, post-maintenance therapy. By contrast, patients with >10 NCN had a very poor prognosis since all these patients finally relapsed [40].

In conclusion, these data suggest that RT-PCR could be used as a complementary assay for the RQ-PCR approach, especially within the subgroup with 1–10 NCN.Furthermore, it is important to note that the relatively high specifity of RT-PCR assay is not reason enough to substitute a highly sensitive, standarized and high through-put technology such as RQ-PCR.

References

1-Warrell RP, de The H, Wang ZY (1993): Acute promyelocytic leukemia. N. Engl. J. Med. 329, 177–189.

2-De The H, Chomienne C, Lanotte M(1990): The t (15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. Nature. 347:558.

3-Soignet SL, Maslak P, Wang ZG(1997):Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med; 339: 1341–1348.

4-Huang CH, Chen WJ, Wu CC, Chen YC, Lee YT (1988): Complete atrioventricular block after arsenic trioxide treatment in an acute promyelocytic leukemic patient. Pacing Clin Electrophysiol; 22: 965–967.

5- Wang ZY and Chen Z(2008): Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 111(5):2505–2515.

6- Lo Coco F, Diverio D, Avvisati G(1999): Therapy of molecular relapse in acute promyelocytic leukemia. Blood. 94:2225–2229.

7- Grimwade D, Howe K, Langabeer S (1999): Establishing the presence of the t (15;17) in suspected acute promyelocytic leukaemia: cytogenetic, molecular and PML immunofluorescence assessment of patients entered into the M.R.C. ATRA trial. M.R.C. Adult Leukaemia Working Party. Br J Haematol ; 94: 557–573.

8-Diverio D, Rossi V, Avvisati G, Pandolfi PP, Biondi A(1998): Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARa fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter ‘‘AIDA’’ trial. GIMEMA-AIEOP Multicenter ‘‘AIDA’’ Trial. Blood 1998; 92: 784–789.

9- Gallagher RE, Yeap BY, Bi W(2003): Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood. 101 7: 2521–28.

10-Cassinat B, De Botton S, Ades L, Raffoux E, Escoffre-Barbe M, Baruchel A(2007): Assessment of PML-RARα real-time quantitative PCR for definition of molecular relapse in APL patients (results of the APL Study Group). Blood (ASH Annual Meeting Abstracts) 14;106-108.

11-Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N (2003): Standardization and quality control studies of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia: a Europe against cancer program. Leukemia; 17: 2318-2357.

12- Cote S, Zhou D, Bianchini A.and Zhou D, Bianchini A, Nervi C, Gallagher RE (2000): Altered ligand binding and transcriptional regulation by mutations in the PML/RARalpha ligand-binding domain arising in retinoic acid-resistant patients with acute promyelocytic leukemia. Blood. 96:3200.

13- Sanz MA, Martin G, Rayon C(1999): A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARα-positive acute promyelocytic leukemia: PETHEMA group. Blood. 94(9):3015–3021.

14-Burnett AK, Grimwade D, Solomon E(1999): Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the Randomized MRC Trial. Blood. 93(12):4131–4143.

15- Fenaux P, Chastang C, Chevret S, *et al.*,(1999): A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia: the European APL Group. Blood. 94(4):1192–1200.

16-Tallman MS, Nabhan C, Feusner JH(2002): Acute promyelocytic leukemia: evolving therapeutic strategies. Blood. 99(3):759–767.

17- Sanz MA, Lo Coco F, Martín G. (2000): Definition of relapse risk and role of non-anthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood. 96:1247–1253.

18- Breccia M, Diverio D, Noguera NI, and Bonnet D(2004): Clinico-biological features and outcome of acute promyelocytic leukemia patients with persistent polymerase chain reaction-detectable disease after the AIDA front-line induction and consolidation therapy. Haematologica. 89:29–33.

19-Jurcic JG, Nimer SD, Scheinberg D(2001): Prognostic significance of minimal residual disease detection and PML/RAR-α isoform type: long-term follow-up in acute promyelocytic leukemia. Blood. 98:2651–2656.

20-Tobal K, Moore HH, Macheta MM, Liu Yin JA (2001): Monitoring minimal residual disease and predicting relapse in APL by quantitating PML-RAR transcripts with a sensitive competitive RT-PCR method. Leukemia; 15:1060-5.

21-Sanz MA, Tallman MS, Lo Coco F(2005): Tricks of the trade for the appropriate management of acute promyelocytic leukemia. Blood. 105:3019-3025.

22-Wells RA, Catzavelos C, Kamel-Reid S (1997): Fusion of retinoic acid receptor a to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. Nat Genet 1997; 17: 109–113.

23-Cassinat B, Zassadowski F, Balitrand N, Barbey C, Rain JD, Fenaux P(2000):Quantitation of minimal residual disease in acute promyelocytic leukemia patients with t(15;17) translocation using real-time RT-PCR. Leukemia ;14:324-8.

24- Gameiro P, Vieira S, Carrara P, Silva AL, Diamond J, De Sousa B(2001) The PML-RAR transcript in long-term follow-up of acute promyelocytic leukemia patients. Haematologica; 86:577-85.

25-Bolufer P, Lo Coco F, Grimwade D, Barragán E, Diverio D, Cassinat B(2001):Variability in the levels of PML-RAR fusion transcripts detected by laboratories participating in an external quality control program using several reverse transcription polymerase chain reaction protocols. Haematologica ;86:570-6.

26- Grimwade D, Howe K, Langabeer S, Burnett A, Goldstone A, Solomon E(2002):Minimal residual disease detection in acute promyelocytic leukemia by reverse-transcriptase PCR: evaluation of PML-RARa and RARa-PML assessmen in patients who ultimately relapse. Leukemia ;10:61-64.

27-Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N (2003): Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia: a Europe against cancer program. Leukemia; 17: 2318-2357.

28-Slack JL, Waxman S, Tricot G(2002): Advances in the management of acute promyelocytic leukemia and other hematologic malignancies with arsenic trioxide. Oncologist 7, 1–13.

29-Carlos Santamaría, Maria Carmen Chillón, Carina Fernández, Patricia Martín-Jiménez, Ana Balanzategui, Ramón García Sanz, Jesús F. San Miguel, Marcos-Gonzalez González (2007):Using quantification of the PML-RARα transcript to stratify the risk of relapse in patients with acute promyelocytic leukemia. Haematologica. Vol 92, Issue 3, 315-322 doi:10.3324.

30-Miguel A. Sanz, M, Pau Montesinos, Chelo Rayón, Alexandra Holowiecka, Javier de la Serna, MDGustavo Milone, MDElena de Lisa, MDSalut Brunet (2010): Treatment of APL Blood. 2010;115(25):5137-5146.

31-Zhou GB, Kim SH, Ding W, Schultz C, Warrell Jr RP, Gallagher RE(2007):Frequent mutations in the ligand-binding domain of PML-RARa after multiple relapses of acute promyelocytic leukemia: analysis for functional relationship to response to all-trans-retinoic acid and histone deacetylase inhibitors *in vitro* and *in vivo*. Blood ; 99:1356–1363.

32-Mandelli F, Diverio D, Avvisati G(1997): Molecular remission in PML/RAR α-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy: Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood. 90(3):1014–1021.

33-Lee S, Beau MM, Bitts S, Davis EM, Kogan SC(2003):Recurring chromosomal abnormalities in leukemia in PML-RARA transgenic mice parallel human acute promyelocytic leukemia. Blood; 99: 2985–2991.

34-Sanz MA, Martín G, González M(2004): Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA Group. Blood. 103:1237-1243.

35-Schnittger S, Weisser M, Schoch C(2003): New score predicting for prognosis in PML-RARA+, AML1-ETO+, or CBFBMYH11+ acute myeloid leukemia based on quantification of fusion transcripts. Blood. 102 8: 2746–55.

36-Gallagher RE, Yeap BY, Bi W(2003): Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood. 101 7: 2521–28.

37-Sanz MA, Martín G, González M(2004): Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA Group. Blood. 103:1237-1243.

38-Gallagher RE, Yeap BY, Bi W (2003): Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood.101 7: 2521–28.

39-Sanz MA, Tallman MS, Lo Coco F(2005):Tricks of the trade for the appropriate management of acute promyelocytic leukemia. Blood. 105:3019-3025.

40-De Botton S, Coiteux V, Chevret S (2004): Outcome of childhood acute promyelocytic leukemia with all-trans-retinoic acid and chemotherapy. J Clin Oncol. 22:1404-1412.

1/8/2013