The Role of ATRA Followed by Chemotherapy in the Treatment of Acute Promyelocytic Leukemia

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Abstract- There are different treatment protocols available for acute promyelocytic leukemia (APL) such as all-trans retinoic acid (ATRA) plus chemotherapy or arsenic trioxide (ATO) based regimens. In this study, we focused on the role of ATRA followed by an anthracycline-containing chemotherapy regimen. This study reported the outcome of APL patients at 501 army hospital; Tehran, Iran. Seventy-three patients were included between 1995 and 2015. Treatment in our center for the majority of cases included induction with ATRA followed by Cytarabine (AraC) and an anthracycline agent (daunorubicin), and then three cycles of consolidation chemotherapy. Maintenance consisted of a 2-year period of medication with ATRA, Methotrexate (MTX) and 6-mercaptopurine (6-MP). Relapsed cases were treated with ATRA and a combination of etoposide, mitoxantrone, and cytarabine. Kaplan-Meier estimate was used to calculate survival rates. We detected 5- and 10-year overall and disease-free survival rates of 51.6% and 50.2% respectively. For those patients who survived induction deaths and received ATRA-based chemotherapy the 5-year OS and DFS rates were 68.8% and 66.5%, respectively. Hematologic complete remission (CR) was observed in all but three patients, and relapse occurred in 12 cases. The cardinal causes of induction death were disseminated intravascular coagulation (DIC) and infection. Up to the end of the follow-up time, 31 patients died including 11 cases of the relapsed disease. The combination of ATRA and chemotherapy could lead to an acceptable CR rate and relapse incidences in newly diagnosed APL patients, but more effective strategies need to be developed for screening and treatment of relapse.

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Introduction

Acute promyelocytic leukemia (APL) is a unique entity among acute myelogenous leukemia (AML) subtypes based on its cytogenetic features, which is a translocation between chromosomes 15 and 17. This very translocation leads to the production of a fusion gene coding PML-RARα protein (1). The frequency of this subtype in previous studies was shown to be about 5 to 20% of all AML cases (2,3). APL was once one of the worst subtypes of leukemia, mainly due to early mortality caused by coagulopathy, but since the advent of all-trans retinoic acid (ATRA) and target specific therapy the overall survival (OS) and disease-free survival (DFS) have been substantially improved(4). There are also several promising results reported in the literature with arsenic trioxide (ATO) for either new (5) or relapsed APL cases (6). Furthermore, combinations of ATO and ATRA along with an additional agent (gemtuzumab ozogamicin (GO) or idarubicin) for high-risk patients, have shown to be beneficial in some recent trials (7).

There is evidence supporting the administration of cytarabine (AraC) in the treatment regimen while others did not due to reduced toxicity observed in regimens without AraC (8). In this study, for the first time in Iran,

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we conducted a survival analysis and prognostic factor evaluation on newly diagnosed APL patients with a treatment including ATRA, AraC plus an anthracycline agent.

Materials and Methods

Between May 1995 and November 2015, all newly diagnosed patients with ages of 15 or more with a diagnosis of acute promyelocytic leukemia at the 501 army hospital, Tehran, Iran were included in the study. Diagnoses were made based on French American British morphological features on bone marrow aspiration and biopsies, flow cytometry and cytogenetic testing for T (15; 17) or testing for PML-RARα fusion gene by polymerase chain reaction (PCR). Values, including CBC at diagnosis, time of complete remission (CR), bone marrow aspiration and biopsy reports and dates of diagnosis, the start of induction chemotherapy, last contacts, relapse, and death (in cases of relapse and death) were extracted from the medical records. CR defined as follows: absolute neutrophil count of greater than 1000/μL, platelet count of greater than 100000/μL, independence from red blood cell transfusion, the absence of extramedullary involvement and a bone marrow blast of less than 5%. Treatment protocol in our center consists of induction with ATRA 45 mg/m^2 followed by AraC 100mg/m^2 (days 1 to 7) and daunorubicin 45 mg/m^2 (days 1 to 3) then three cycles of consolidation chemotherapy with AraC plus daunorubicin with the same dosage for each 21 days followed by a maintenance therapy, including a 2-year period of ATRA 10 days each month in addition to methotrexate and 6-mercaptopurine. Relapses were treated by a combination of ATRA and chemotherapy, including etoposide 100 mg/m^2 for five days, mitoxantrone 12 mg/m^2 for three days and AraC 100 mg/m^2 for five days. It should be noted that a minority of the patients did not receive ATRA before the year 2002 and after a primary survival analysis, they were dropped out from the rest of analyses. Treatment protocol is summarized in table 1.

Table 1. The treatment protocol

Treatment	Details	Dosage and timing
Induction	ATRA	45 mg/m^2
	AraC	100mg/m^2 (Day1-Day7)
	Daunorubicin	45 mg/m^2 (Day1-Day3)
Consolidation	AraC	100mg/m^2 (Day1-Day7)
	Daunorubicin	45 mg/m^2 (Day1-Day3)
Maintenance	ATRA	45 mg/m^2 (10 days per month)
	MTX	20 mg/m^2 oral weekly
	6-MP	60 mg/m^2 oral daily
After relapse	ATRA	45 mg/m^2 (Day1-Day5)
	etoposide	100 mg/m^2 (Day1-Day5)
	mitoxantrone	12 mg/m^2 (Day1- Day3)
	AraC	100 mg/m^2 (Day1-Day5)

Management of DIC included rapid initiation of ATRA and transfusion of platelets and fresh frozen plasma (FFP) in order to rectify and maintain the platelet count and plasma fibrinogen level. Patients with differentiation syndrome were treated with IV dexamethasone. Informed consent was obtained from all patients to use their medical records as a resource for research. The study was approved by the ethical committee of AJA University of medical sciences.

The data were retrospectively analyzed, and Kaplan-Meier estimates were used to calculate the survival rates. Comparisons made with the log-rank test. Univariate analysis was done for identifying hazard ratios of potential prognostic factors based on a Cox proportional hazard model. Multivariate analysis was not performed because of the diversity of studied patients. P-values of less than 0.05 were considered significant. All statistical analyses were performed using R software for windows version 3.3.2. “Survival” and “etm” packages were used.

Results

Seventy-three cases were included in the study. Mean age was 34.58±14.32 years. 56.2% were male

(n=41), and the remaining 43.8% were female (n=32). Median follow-up time was 22 months (with a maximum of 242 months).

Basic characteristics of studied population are summarized in table 2.

Table 2. Basic characteristics of studied population

Covariate		Frequency
Sex	**Male**	56.2% (n=41)
	Female	43.8% (n=32)
APL type	**De novo**	97.26% (n=71)
	Treatment related	1.37% (n=1)
	Evolution from MDS	1.37% (n=1)
Induction regimen	**ATRA based**	91.78% (n=67)
	Chemotherapy only	6.85% (n=5)
	ATO based	1.37% (n=1)
Differentiation syndrome	**Yes**	5.5% (n=4)
	No	94.5% (n=69)
Outcome	**Alive**	57.5% (n=42)
	Dead	42.5% (n=31)
Remission	**Complete remission**	95.89% (n=70)
	Partial remission	2.74% (n=2)
	Refractory	1.37% (n=1)
Relapse	**Yes**	16.44% (n=12)
CBC at presentation	**Mean WBC**	13600±18300 /µL
	Mean hemoglobin	8.84 ±1.84 g/dL
	Mean platelet	59290±61500/µL

Chemotherapy protocol was performed as cited before for all except six patients; five patients received only chemotherapy regimen (without ATRA) since lack or difficulty of accessibility or cost issue of ATRA in our country before 2002; and also one patient had had the diagnosis in another center and received arsenic trioxide (ATO) induction therapy but was resistant to treatment and referred to our center with a WBC count of 100000 /µL. Hematologic complete remission (CR) was achieved during a median time of 45 days for all patients who survived induction chemotherapy except three cases (4.11%); 2.74% (n=2) achieved partial remission, and 1.37% (n=1) were refractory. Relapse occurred in 16.44% of cases after complete remission (n=12) including one case of CNS relapse (8.33% of relapses) and 11 bone marrow relapse (91.67%). One patient from the relapsed group has undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a full matched sibling donor after the second complete remission (CR2) and is still alive, three cases went to CR2, but they had the second relapse and died from which one case had been referred for allo-HSCT. The other eight patients died after the first relapse due to complications of the disease or treatment. Five, 10- and 16-year cumulative incidence of relapse was 25.64% (95%CI: 14.52%-42.82%) among the patients (Figure 1).

A total number of 31 patients (42.5%) died during the follow-up time of which 20.55% (n=15) were before one month, 9.59% (n=7) were before one week and 1.37% (n=1) before induction. Those seven patients who died during induction with ATRA did not survive enough to receive chemotherapy. Causes of all-time mortality are summarized in table 3.

Table 3. Causes of death

Causes of death	Frequency
DIC	11
Relapse or Refractory Disease	11
Infection	6
Other causes	3
Total	N=31

It should be cited that the cardinal cause of early death (less than one month) remained DIC. The category "Other causes" of death included advanced breast cancer (n=1), massive hemoptysis due to active cavitary

tuberculosis (n=1) and cardiac arrest due to hyperglycemia and hyperkalemia (n=1).

Survival analysis on all patients showed 5, 10- and 16-year overall survival and disease-free survival rates of 51.6% (95% CI: 38.4%-63.3%) and 50.2% (95% CI: 37.1%-62.0%) respectively. Estimated 5, 10- and 16-year OS and DFS rates for cases who were treated with ATRA-based chemotherapy and survived induction were 68.8% (95% CI: 51.2%-81.1%) and 66.5% (95% CI: 48.9%-79.2%) (Figure 2).

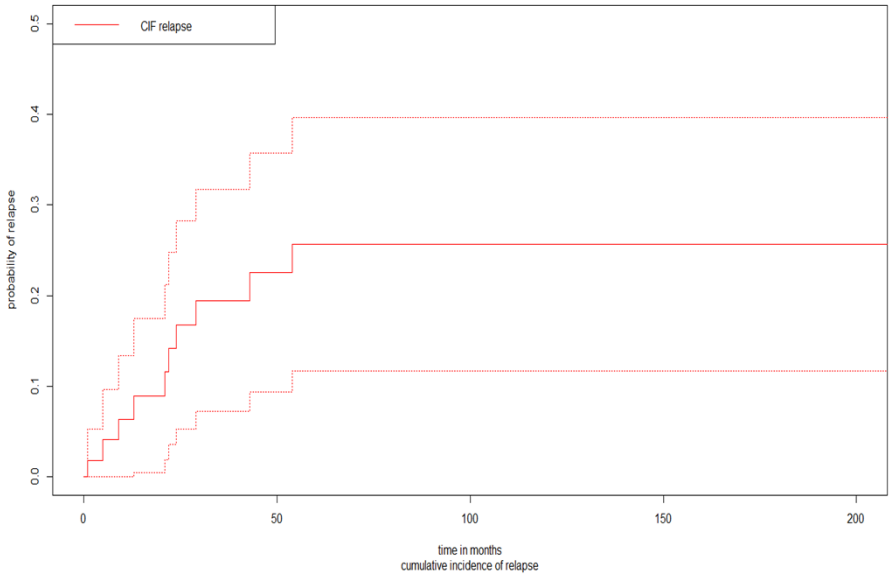

Figure 1. Cumulative incidence of relapse (CIF: cumulative incidence function.)

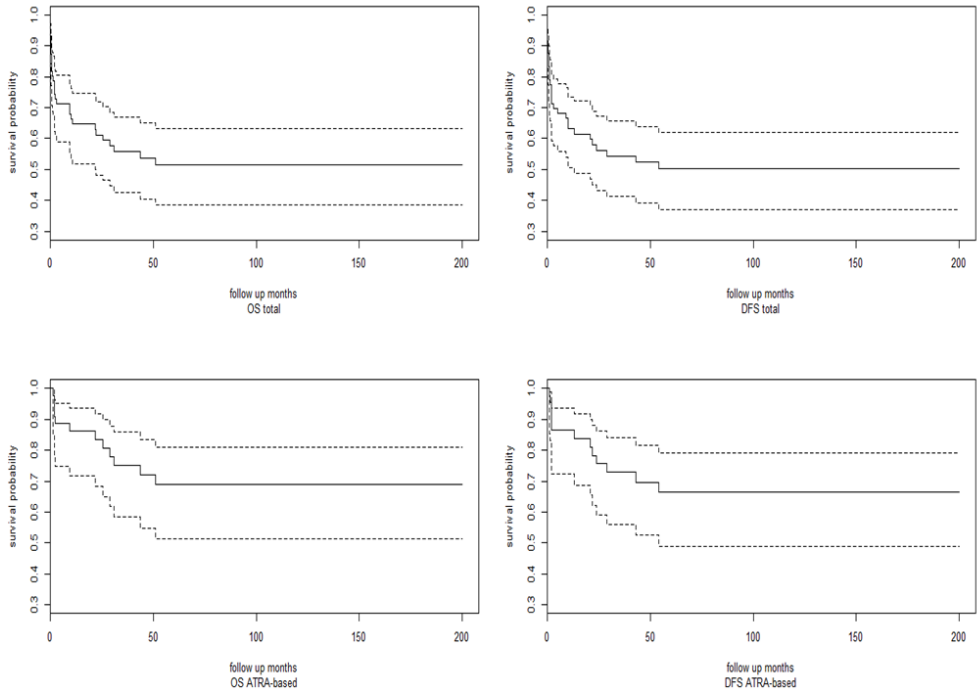

Figure 2. Overall and disease-free survival of patients in total and ATRA-based induction group

Ages of more than 60 and induction regimens without ATRA are significantly different from ages less than 60 and induction with ATRA (log-rank test, *P*=0.04 and <0.001, respectively) (Table 4).

Table 4. The effect of age and induction on outcome

Prognostic factor		5-year overall survival	*P*
Age	**≥60 (N=5)**	20.0%	0.04
	<60 (N=68)	54.4%	
Induction with ATRA plus chemotherapy		65.1%	<0.001
Induction with conventional chemotherapy		20.0%	

While the mean WBC count is higher among the dead group and the mean platelet count and hemoglobin levels are lower, but the univariate analysis did not show their effect on the outcome (Table 5).

Table 5. Hazard ratio of CBC elements based on univariate analysis and comparison of means

CBC	Mean		t-test *P*	Hazard ratio	*P*	95% Confidence Interval
WBC	Alive	11910 /µL	0.47	1.00	0.15	1.00-1.00
	Dead	15492 /µL				
Hemoglobin	Alive	9.31 g/dl	0.03	0.85	0.18	0.67-1.08
	Dead	8.22g/dl				
platelet	Alive	78866 /µL	0.01	1.00	0.08	1.00-1.00
	Dead	35800 /µL				

Discussion

The primary aim of this study was to evaluate the efficacy of ATRA and chemotherapy protocols consisting of AraC plus anthracycline agents followed by a maintenance therapy in primary APL on a single institution basis. We reported 5-year overall and disease-free survival rates of 51.60% and 50.1% respectively, which is lower than expected for APL patients in comparison to other centers using combination therapies with ATRA and ATO in either consolidation (9) or both induction and consolidation like Liu YJ *et al.*, study on 340 patients in a single center experience which reported a 5-year OS of 89% (10). This lower OS rate could be related to heterogeneity of our studied population. Number of 501 army hospital is a referral army hospital in Iran and patients from all over the country were admitted into this hospital, hence may be there are more high-risk patients and also an undesirable delay in the start of treatment in our sample group than other general hospitals. Furthermore as previously cited we had patients in our cohort with maximum ages of even 92 years old. Moreover, a fraction of our patients did not receive any kind of target specific therapy and treated only with conventional chemotherapy. These obstacles obviously caused a steep decrease in the detected survival rate; excluding the latter group (chemotherapy without ATRA) our reported 5- and 10-year OS would become 65.1%.

Our results also revealed that addition of ATRA to induction chemotherapy had a significant impact on survival rates, which is similar to study of Kanamaru A *et al*., for Japan Adult Leukemia Study Group (11). According to the Japanese study which was a multicenter trial on 110 patients with a median follow-up of 21 months addition of ATRA to induction therapy could lead to significantly higher CR and lower mortality rates.

Early mortality in our series of patients was 20.55%. This rate is compatible with previous studies specially those outside of clinical trial settings. The multicenter study of Park JH *et al.*, in 1400 APL patients revealed an overall early death of 17.3% with a slight decline in the past two decades but not a significant drop (12).

Our treatment protocol resulted in an acceptable CR rate in comparison to clinical trials using similar protocols; for instance, European APL group which reported a 92.5% CR rate (13). This CR rate is mainly attributable to the early addition of AraC and chemotherapy to ATRA in the induction regimen based on previous results like Ades L *et al*., study, which had shown the benefits of adding AraC to induction in terms

of survival, CR rate and relapse incidence (14). Although relapse incidence in our study was almost similar to what experienced in earlier studies such as Clavio M *et al.*, on 91 APL patients who have reported 13% relapse rate (15) but the mortality rate after the first relapse was much higher than former experiences like European APL group study.

It should be considered that some studies like the single center experience of Ruiz-Arguelles GJ *et al.*, in 14 patients Mexico reported resistance to ATRA in relapsed cases (16). Also it has been shown in the study of Esteve J *et al*., that the hematological relapse; so is the recruited criteria in our study; compared to molecular relapse is associated with a significantly poorer outcome (5-year OS 24% vs. 64%) (17) so it can be deduced that use of molecular markers to identify relapse before development of hematological abnormalities might be of outstanding benefit, and it may be one of the reasons for treatment failure in relapsed cases in our study.

As mentioned before the mean WBC count are higher among the dead patients and also the mean platelet count and hemoglobin concentration are lower which is in line with the literature, but we could not be able to detect a significant link between CBC elements (neither WBC count, Hb nor platelet count) and outcome of the treatment may be as a result of the vastly diverse studied population and presence of other factors affecting outcome, which could not be properly adjusted and stratified. Major causes of induction death in our study were DIC and infection, which are comparable to previous studies such as European APL group experience and also Avvisati G *et al.*, multicenter study on 995 participants (13,18)..

It seems that ATRA based induction regimens in addition to maintenance therapy should be considered in terms of acceptable remission and low relapse incidences. It also should be considered that more efficient policies for diagnosis and treatment of relapsed cases need to be developed.

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