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ORIGINAL ARTICLE

Effects of Clinical, Haematological and Immunophenotyping Factors on the Prognosis of Acute Promyelocytic Leukaemia (APL) at the Tabriz Haematology and Oncology Research Centre.

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ABSTRACT

Background: Acute Promyelocytic Leukemia is one of AML subgroups that is high lighted by coagulopathy and different mode of treatment. It is specified by district cell morphology and immunophenotyping characteristics.

Aims: The aim of this study was evaluation of the effects of cytologic , clinical and biologic factors specially CD34 expression in prognosis in APL patients.

Materials and Methods: In a Descriptive retrospective analysis the files of 60 APL patients reviewed and the extracted data's statistically analyzed using SPSS soft ware with Chi Square and T- test.

Results: Dislike references complete remission and disease free survival (DFS) had no significant correlation with age, sex, WBC, Hb, platelet count, purpura, CD34 status, and percent of Blasts in bone marrow. There was no significantly statistical correlation between CD34 expression with morphology, age, sex, WBC, platelet count, percent of BM blasts and purpura. Cases with CD34 expression had sever anemia (5.8± 1.08) in comparison with CD34 negative APLs (P=0.020).

Conclusion: In spite of the influence of known prognostic factors on prognosis, results of our study were not concordant with references, therefore it is logical to think that in APL there should be different prognostic factors .Failure in obtaining complete remission in all $CD34^{+}APL_{s}$ cases may be the cause of poor prognosis of CD34 positively in these patients and needs further studies for better clarification.

Key Words : Acute Promyelocytic Leukemia, CD34, Complete Remission, Disease Free Survival.

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Introduction

Acute Promyelocytic Leukaemia (APL) is characterized by typical promyelocytes and coagulopathy that result in severe haemorrhage, which accounts for about 10% of adult acute myeloid leukaemia [1]. It is the cause of 46-80% early deaths [2],[3],[4], and is characterized by the Promyelocytic Leukaemia-Retinoic Acid Receptor α(PML-RAR α) fusion gene [1].Besides cellular morphology, genetic translocation t(15;17)(q22,q12) and specific immunophenotyping ($CD13^{+}$,$CD33^{+}$,$CD14^{-}$,$CD9^{+}$,$HLADR^{-}$,$CD4^{-}$) are necessary for confirming diagnosis (2,4,5,6) Most of the cases developed excellent prognosis after they entered complete remission (CR) (85-90%) using All-Trans Retinoic Acid(ATRA) with or without antracyclin chemotherapy [7]. It is believed that different clinical and biological parameters such as age, number of White Blood Cells (WBC) and purpura affect Complete Remission and survival rate. A lower age group (<30 years), a WBC

count of <1000/µl, and absence of purpura have a positive effect on early development of CR and higher survival [7],[8]. Pancytopenia is the most common finding in APL, although the white blood cell count was elevated in 10-30% of patients [9]. Moreover, recent studies reported that CD34 surface expression is associated with poor clinical outcome in patients with APL. Despite all these data, the issue of CD34 expression in APL remains unsolved [10].

Regarding the high incidence of APL in our district [11], with respect to the possible ecological issues on its biology and different therapy responses (personal observation), we studied CD34 expression on promyelocytes of APL cells, its relationship with complete remission and disease free survival (DFS) rate, and other clinical factors (except cytogenetics) as prognostic factors, at the Haematology and Oncology Research Center of the Tabriz University of Medical Sciences.

Materials and Methods

In a cross-sectional retrospective analysis study, we reviewed the files of 60 APL patients. Forty two cases had a complete flowcytometric analysis (acute leukaemia panel of our center).

Flowcytometry was done from bone marrow aspirates by the FACS caliber, Becton Dickenson apparatus. Number of white blood cells, platelets, and haemoglobin levels have been recorded from the patient's blood on the day of their admission, by using H1(Bayer) automated cell counter . The cell morphology and percent of Bone marrow Promyelocytes (Blasts) were obtained from the patient's bone marrow aspiration cytological reports. Other demographic and immunophenotyping information, as well as CD34 expression, got special attention.

For the evaluation of DFS, the time between complete remission till relapse, or the last follow up that showed complete remission status or death from unrelated causes, was an important criterion. The role of age, sex, WBC, platelet, count, haemoglobin rate, percent of marrow blasts, purpura, and CD34 expression in remission-induction (CR) and DFS were analyzed. The relationship between CD34 expression and the same factors were also evaluated. Any percentage expression of CD34 was recognized as positive in our cases.

According to age and WBC count, the patients were divided into group A (more or less than 30 years), group B (more or less than 60 years), and WBC more or less than 10000/ µl, respectively [2].

Regarding platelet count, patients were divided into those with less or more than 20000/µl. A Haemoglobin level of 10 gr/dl was also considered as analytical range (threshold). Datas were analyzed statistically by SPSS13 software after extraction from patient's files, and Khey 2 and T tests were used for statistical analyzing the datas correlated with CR and DFS.

Since 11 of 60 cases died before starting treatment, they were not considered for evaluation of the above mentioned factors with CR .We analyzed this part of the study only for 49 patients, and because only 28 cases had reached CR and DFS, the correlation between the mentioned factors in the checklist was evaluated for 28 patients, and $p<0.05$ was considered significant.

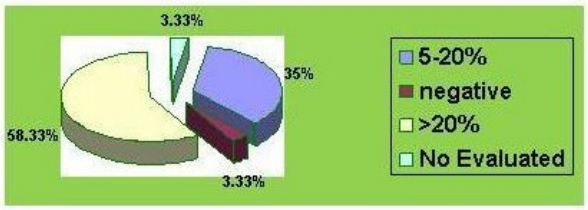
Table/Fig 1: The Personal Characteristics of the APL Patients

Characters		Number	Percentage(%)
Age:		23	38.3
Group A	< 30 Years		
	= 30 Years	37	61.7
Group B	< 60 Years	56	93.3
	= 60 Years	4	6.7
Hemoglobin Rate	< 10 g/dl	56	85
	= 10 g/dl	51	15
WBC	<10000 /µl	9	81.7
	=10000/µl	49	18.3
Plt	<20000/µl	16	26.7
	=20000/µl	44	73.3
Purpura	Positive	38	63.3
	Negative	22	36.7
Blast in BM	<70%	3	5
	=70%	57	95
Morphology	Hypogranular	2	3.3
	Hypergranular	58	96.7

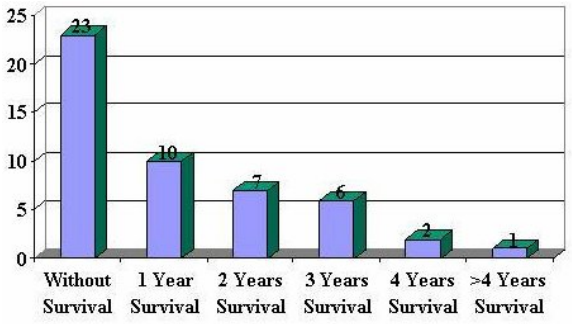
Results

Of the sixty patients evaluated, 34 (56.7%) were males and 26 (43.3%) were females. Cases were in the range of 11-71- year- old, and mean age was at 33.63.The highest age related to incidence, was 30-39 years. [Table/Fig 1] shows the characteristics of the studied cases. Of 39 patients that were checked for CD34, 2 cases (5.1%) were considered positive regarding 20% severity counting as cut off point, and 4 cases (10.3%) were considered positive without considering any

cut off point [Table/Fig 2]. Only one out of 4 CD34+ patients had hypogranular morphology, and from 49 cases treated, 28 patients (57.1%) entered remission, and 11 patients who had died before starting treatment were not considered in the statistical analysis.



Table/Fig 2: Distribution of CD34 in APL patients



Table/Fig3: Survival values of 49 Under Treatment APL Patients

[Table/Fig 3] shows the survival range of 28 patients' conjunctions with CR rate.DFS was estimated least and last 1 and 63 months respectively. As a mean they had 18.76 months DFS.

Of 28 cases who entered CR, 16 were males and 12 females (P=0.777).Mean DFS was 16.42 ± 4 months (P=0.495). All of the cases with CR were under 60-year-old (P=0.179), and the mean DFS was 18.76 ± 2.9 months [Table/Fig 4]**.**Nineteen patients with CR (67.9%) had purpura or overt bleeding at presentation (P=0.553), and their mean DFS was 16.84 ± 2.8 months (P=0.347).Of 19 CR patients, 3.6% had marrow blast count less than 70%, and the rest (92.4%) had marrow blast count equal or more than 70%(P=1.000) and the mean DFS was 19.39 ± 2.9 months (P=0.276).None of the 28 cases that entered CR showed CD34 expression, and of those 21 cases that did not enter CR , 3 cases (18.8%) expressed CD34 positivity (P=0.086).

One of the $CD34^+$ cases died before starting of treatment, and so we omitted it from the statistical analysis in this part of the study. The mean DFS of CD34 negative patients was 16.15± 3.05 months.

Twenty seven out of 28 patients who entered CR, had hypergranular morphology (P=1.000), and the mean DFS was 20.53±4.3 months. Regarding the only case in CR with hypogranular morphology, who had DFS of 7 months, there was no significant statistical difference (P=0.448).

Results of CD34 positivity in relationship with cell morphology and other factors:

Because CD34 expression was checked only in 39 cases, we considered only these 39 cases for statistical analysis in this part [Table/Fig 5]. Three out of 4 CD34+ (10.25%) were hypergranular (P=0.197).

Table/Fig 4: Characteristics of 28 patients in Complete Remission

	Total 28 case		Pvalue
	n=13	n=15	
Age(years)	<30	>30	0.247
Mean DFS(months)	16.53±4	20.70±4.2	0.487
	n=22	n=6	
Hemoglobin (gr/dl)	<10	>10	0.214
Mean DFS (months)	19.93±3.6	14.50±3.2	0.455
	n=24	n=4	
White Blood Cells (/µl)	<10,000	>10,000	0.688
Mean DFS (months)	20.92±3.2	9.62±3.9	0.206
	n=20	n=8	
Platelets count (/µl)	>20,000	<20,000	-
Mean DFS (months)	19.85±3.3	16.06±6.1	0.567

Table/Fig 5: Findings in CD34+ and CD34- patients

	CD34+	CD34-	P value
Mean Hemoglobin	lower	higher	**0.020**
WBC Count	"	"	**0.541**
Plt Count	"	"	**0.572**
Bone Marrow Blasts	87.5±2.5	84±2.1	**0.587**
Mean Age	38.75±10.5	34.09±2.4	**0.550**

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Discussion

Many biological and clinical factors are involved in the prognosis of APL patients, from the point of remission induction and relapse rate. It is believed that gender plays a prognostic role, and that the prognosis is poor in males[2].Twenty eight out of 49 treated patients entered complete remission in our study. Sixteen of these (57.1%) were males. This is the similar to the results of a study of 47 cases with female dominancy, but without any significant statistical differences [2]. Older age (>60 years) plays a negative role in prognosis of AML patients [12]. All the patients who had entered CR were less than 60 years old in this study.

It is thought that a platelet count of less than 40,000/µl at presentation is a favorable prognostic factor with respect to relapse [14]. Twenty out of

28 patients who entered CR (71.4%) in our study had a platelet count of more than 20,000/µl, that was not significant from statistical point of view. The same results were obtained from a study which was trying to show the correlation between CD34 positivity and CR and platelet level in APL patients [2] . DFS was also low in patients with platelet counts less than 20,000/µl but statistical analysis did not show any significant difference between cases with higher and lower than 20,000/µl platelet counts and DFS. Some studies stressed that lower platelet count at diagnosis is related to early and consequent lower DFS [2] .

In spite of some studies we could not show any significant correlation between WBCs and CR. Meanwhile, there was no correlation between appearing purpura and CR in our patients, but in a study with 196 APL patients, the absence of purpura or fine purpura was considered as a favorable factor in the development of CR (P=0.461), [13].

In most studies, expression of CD34 on the surface of APL cells was considered as a poor prognostic factor[15],but there are also some studies that could not addressed it [15],[16]. However, there were no statistically significant differences in the other clinical parameters, such as age, haemoglobin level, WBC , platelet count between the CD34+ and CD34- groups in the Albano et al study. On the other hand, they didn't find any differences between the two groups in terms of complete remission, overall survival, and disease-free survival [10].

Expression of CD34 on APL cells also has been considered as a poor clinical prognostic factor [2] .There are also some data reporting the poor prognostic correlation of CD34 positivity with leukocytosis and other concomitant poor prognostic clinical factors [17]. Another study showed that the high WBC count (>10,000) at presentation is related to overall survival [17]. .In our study, 4 out of 39 cases(10.3%) expressed CD34 on their promyelocytes, but one of which died before starting treatment. None of the 28 patients who entered CR expressed CD34.These findings indirectly show the poor prognosis related to the CD34 expression on APL cells. Still it is believed that expression of CD34 in APL may be associated with poor prognosis because of its correlation to higher WBC counts [14]. In this study, CD34+ patients had lower WBC counts which differed from the results of other studies (2,10,14) . On the other hand, cases with WBC<10,000/µl showed longer DFS.

The age group less than 30 is normally considered as a favorable factor [13].The results of a study on 196 APL patients showed longer DFS in patients younger than 30 years (P=0.0003),but in our study, the patient's lower age group (<30-year-olds) was related to lower DFS . Males with CR had mean DFS better than the females, but there was no statistical significance among them. Despite our study, results from Taiwan stated that male cases had poor prognosis than female ones [18]. The CD34 positivity in males and females was also evaluated in this study, but it did not show any significant difference.

There was no significant statistical correlation between purpura and obvious clinical haemorrhage with DFS, but all patients showing purpura or haemorrhage at presentation, had lower DFS. Studies show that absence of purpura is a favorable factor in remission induction and rate of survival [2]. It seemed that absence of purpura had a positive effect on the longevity of DFS ,but lack of studies in this regard, makes it necessary to do further studies to clarify it.

Regarding the outcome of the research,, correlation of hypogranularity and CD34 expression is statistically significant [2],[19]. Regarding the low number of CD34+ patients and absence of CR in this patients group in this study, we could not get any statistical or clinical clue for evaluating this parameter. The hypogranular morphology also did not show significant statistical correlation with CR.

Regarding the death of all CD34+ patients, it is logical to consider it as an unfavorable prognostic factor, which needs more studies with higher sample size to be clarified. This consideration is based on results of a study that involved 47 APL cases, and showed CD34+ patients had shorter DFS (P=0.0051).In a cohort study, it was shown that the early mortality rate of the CD34+ patients was 50%,but in spite of the heavy link between CD34 positivity and leukocytosis, there was not significant difference between CD34 positivity and overall survival [17].

Conclusions

Non-significant statistical correlation in most prognostic parameters in this study, may be because of small sample size. However it could be also related to a different natural history of APL in our district, or as an effect of race or other ecological factors.

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