ORIGINAL ARTICLE

Diagnostic Usefulness of AgNOR Scores In Acute Myeloid Leukemia

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ABSTRACT
The silver colloid technique for nucleolar organizer regions (AgNORs) was applied to bone marrow smears in 10 cases of normal morphology marrow and 15 cases of acute myeloblastic leukemia. AgNORs were recorded in the form of 'clusters' and 'dots' in the myeloblasts. Leukemic blasts contained a significantly lower number of AgNOR 'clusters' as compared to normal myeloblasts. The method has the potential of helping in diagnosis of AML and should be adopted while evaluating bone marrow smears.

INTRODUCTION
Leukemias are a group of disorders characterized by malignant proliferation of blood cell precursors [1]. The malignant proliferation in these cancers is clonal in nature and the accumulation of tumour cells eventually crowdes out and displaces the normal haemopoietic residents in the bone marrow and often threatens vital structures elsewhere. The malignant cells never stop dividing, mature slowly and incompletely, their cell cycle time is prolonged and most of these poorly differentiated cells survive longer than normal[2,3] . Leukemias are classified as acute and chronic according to the clinical course and as lymphoid and myeloid according to the cell line predominantly involved[4].

Acute myeloid leukemia is a hetrogenous group of malignancies that appears to share in common, an origin in the cells of the haemopoietic progenitors and affects all age groups. Morphology, cytochemical stains and immunophenotyping are the present day tools for accurate characterization of leukemic cells, which is mendatory for determinig therapy and evaluating prognosis[5].

Various methods are available for the measurement of proliferative rates in tumours, including mitotic counts, estimation of the fraction of cells in s-phase of the cell cycle and immunohistochemistry / immunocytochemistry of proliferation associated antigens / proteins[6]. In addition to these methods , people had been in search of a simple, reproducible and cost effective method. Having this purpose in mind, visualization of Argyrophilic Nucleolar Organizer Regions (AgNORs) by silver colloid method has been much attempted, the history of which dates back to Goodpastuer and Bloom[7] and Crocker and Nar[8] . Bukhari et al[9] applied this silver colloid technique to astrocytomas while, Ahmed et al[10] applied the technique to breast cancers and recommended the use of AgNOR counts / dispersion in diagnosis of these cancers. Fontese et al[11] had used this method to evaluate exfoliative pap smears and tongue in smokers and non-smokers while, Ruz et al[12] applied the technique in oral cancers. Hossain et al[13] concluded that AgNOR analysis was a simple, sensitive and cost effective method for differentiating benign from malignant thyroid swellings. Avdalyan et al[14] found that AgNOR count had a prognostic value in uterine leiomyosarcomas. Thus there is hardly any tissue which has not been evaluated by AgNOR quantification , in an attempt to differentiate benign from malignant lesions and evaluating prognosis . Very few examples of application of this technique to normal and leukemic bone marrows, are available[16].

The aim of the present study was to evaluate the usefulness of AgNOR scores in diagnosis of acute myeloid leukemia (M1 and M2 of FAB classification).

MATERIALS AND METHODS
Two groups were made.

Group-A : Ten bone marrow smears from same number of patients were studied. Smears showing good maturation of all the cell series, having normal number of lymphocytes, plasma cells, and showing no evidence of malignancy were declared 'Normal Morphology Marrow' and were included in Group-1.

Group–B : Fifteen bone marrow smears from same number of patients of AML (M1 and M2 of FAB classification) were included and placed in group B.These patients were diagnosed as acute myeloid leukemia(AML) on the basis of morphology and sudan black / MPO positivity.

AgNOR Staining:

After drying , fresh smears were fixed in absolute ethanol for 20 minutes and saved. These fixed and saved smears were stained with AgNoR method

soon after the diagnosis was confirmed. The smears were incubated in inverted position in a silver nitrate solution as described by Crocker and Nar[8] and adopted by Grotto et al[16] , for 30 minutes, at room temperature under safe light conditions.In this method the silver gets bound to NOR associated proteins which are stained dark brown to black.

Enumeration of NORs :

The AgNOR stained smears were examined under the microscope using 100X lense. The method of Grotto et al(1991) was adopted and AgNORs were recorded in the form of 'Clusters' and 'Dots' in each blast cell. An aggregate of AgNORs within light matrix was counted as 'cluster' while small dots scattered throughout the nucleoplasm were taken as 'dots'. Clusters and dots were recorded in 20 consecutive blast cells in each case of normal and leukemic bone arrow.

Statistical Analysis :

The mean number and standard deviation of 'clusters' and 'dots' in myeloblasts of individual cases was calculated and then the combined mean and standard deviation were calculated for the relavent blast cell (Normal or Leukemic). The significance of differences between the mean numbers of 'clusters' and 'dots' in each case were calculated using student's T test.

RESULTS

The mean number of 'clusters' and 'dots' in twenty consecutive myeloblasts in each case of normal morphology marrow (Fig-1) is shown in table-1.

Fig.1 & Fig.2: The combined mean for 'clusters' in normal morphology marrow was 3.22 +_ 0.77 and for 'dots' it was 0.75 +_ 0.65 .

Table 1:

Case N0	'Clusters'	'dots'
N1	3.70 +_ 0.90	1.20 +_ 0.74
N2	2.93 +_ 0.67	0.66 +_ 0.59
N3	2.95 +_ 0.66	0.60 +_ 0.58
N4	3.05 +_ 0.95	0.56 +_ 0.60
N5	3.55 +_ 0.78	0.85 +_ 0.57
N6	3.15 +_ 0.73	0.91 +_ 0.61
N7	3.15 +_ 0.73	0.90 +_ 0.77
N8	2.90 +_ 0.77	0.50 +_ 0.59
N9	3.07 +_ 0.74	0.85 +_ 0.75
N10	3.70 +_ 0.74	0.52 +_ 0.76
Xc +_ Sc	3.22 +_ 0.77	0.75 +_ 0.65

The mean number of 'clusters' and 'dots' in consecutive twenty leukemic blasts in each case (Fig.2) is shown in table-2.

The combined mean for 'clusters' in leukemic blasts was calculated as 2.29 +_ 0.78 and for 'dots' it was 0.77 +_ 0.70 . The difference between the 'cluster' content of leukemic myeloblasts(Group B) and those of normal myeloblasts(Group A) was highly significant ($p < 0.001$), while the difference between 'dots' of these two cells was not significant ($p > 0.1$). This is depicted in Table-3.

Table 2:

Case N0	'Clusters'	'dots'
M1	2.32 +_ 1.06	0.86 +_ 0.72
M2	2.20 +_ 0.70	0.66 +_ 0.71
M3	1.98 +_ 0.64	1.02 +_ 0.84
M4	2.30 +_ 0.84	1.08 +_ 0.79
M5	2.42 +_ 0.80	0.98 +_ 0.64
M6	2.12 +_ 0.79	0.92 +_ 0.77
M7	1.92 +_ 0.64	0.65 +_ 0.62
M8	2.04 +_ 0.72	0.70 +_ 0.70
M9	2.28 +_ 1.04	0.82 +_ 0.80
M10	2.68 +_ 0.78	0.70 +_ 0.60
M11	2.86 +_ 0.84	0.74 +_ 0.74
M12	2.99 +_ 0.84	0.45 +_ 0.45
M13	2.72 +_ 0.87	0.60 +_ 0.69
M14	1.76 +_ 0.61	0.50 +_ 0.67
M15	2.77 +_ 0.81	0.92 +_ 0.83
Xc +_ Sc	2.29 +_ 0.78	0.77 +_ 0.70

Table 3:

Myeloblast Group	'Clusters'	'dots'
Normal morphology Marrow (Group A)	3.22 +_0.77	0.75+_ 0.65
A M L (Group B)	2.29 +_ 0.78	0.77 +_ 0.70
Group A vs Group B	$p < 0.001$	$p > 0.1$
	(highly significant)	(not significant)

DISCUSSION

Myeloid leukemias are disorders in which there is unregulated clonal proliferation of myeloid stem cells. Advanced and sophisticated techniques like immunophenotyping and cytogenetic analysis are being utilized for diagnosis of haematological malignancies and the quest for an easy, simple, reproducible and relatively cheaper method is still underway. The silver staining of nucleolar organizer regions(AgNORs) is relatively new addition in the list of diagnostic modalities currently available.

The mean number of AgNOR 'clusters' in normal myeloblasts(Group A) was 3.22+_0.77 and

those of leukemic blasts(Group B)was 2.29+_0.78. The mean nuber of 'dots' in normal myeloblasts and leukemic myeloblasts was 0.75+_0.65 and 0.77 +_ 0.70 respectively. These facts are depicted in Fig.3.

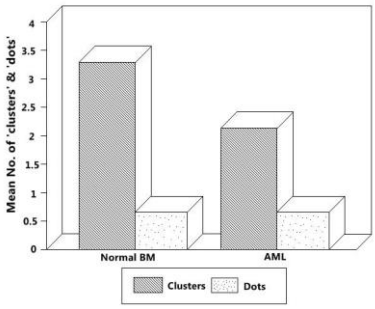

Fig 3:

It has been demonstrated that the number of AgNORs is inversely related to the cell cycle time[15] (Drenzini and Trere 1991).This suggests that a faster cell cycle is associated with a higher number of AgNORs. The cell cycle in leukemic blasts is generally longer than the normal myeloblasts[16,17] (Grotto et al 1993, Ali I et al 2000). The present observation of a significantly lower mean number of AgNOR 'clusters' in leukemic myeloblasts is in agreement with findings of Grotto et al. Grotto et al[16] (1993) described that AgNOR 'dots' vary indepnendently of 'clusters'. The same was observed in present study.

Observations presented uptil now indicate that this technique can help in the diagnosis of acute myeloid leukemia and it also aids in the better understanding of cell kinetics in haematologic diseases. Further exploration is needed to evaluate its usefulness as a diagnostic tool and prognostic marker in acute myeloid laukemias.

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