

ACUTE PROMYELOCYTIC LEUKAEMIA — A STUDY OF SEVEN CASES

R CHERIAN MD, FRCPA*

AND

J BOSCO MBBS, FRACP**

Summary

Seven patients with Acute Promyelocytic Leukaemia (APL) are described, with emphasis on morphology, cytochemistry, ultrastructure and coagulation changes. Six patients were classified as typical hypergranular APL while one patient had the microgranular variant of APL. Criteria for diagnosis and classification are discussed and recent literature is reviewed.

INTRODUCTION

Acute Promyelocytic Leukaemia (APL), a form of acute myelogenous leukaemia is designated by the French-American-British co-operative group as M3 and is diagnosed when the great majority of cells in the bone marrow are abnormal promyelocytes with a characteristic pattern of heavy granulation.¹ The nucleus varies greatly in size and shape and is often bilobed or reniform. Cells containing bundles of Auer rods (faggot cells) add to the characteristic morphology. Clinically, the condition is associated with a high incidence of disseminated intravascular coagulation,² and, chromosomal analysis has revealed a specific abnormality: $t(15q+17q-)$.³

Recently, a morphological variant has been described, termed the "microgranular variant" or M3 variant⁴ in which the majority of cells on light microscopy lack the characteristic coarse granulation, but exhibit nuclear lobulation or segmentation and can therefore pose a diagnostic problem. The cytogenetic abnormality, ultrastructure and predisposition to disseminated intravascular coagulation are unchanged. More recently, a smaller subgroup has been identified — "the hyperbasophilic variant" in which there is a predominance of small promyelocytes with markedly basophilic cytoplasm.⁵

The purpose of this study is to analyse seven cases of Acute Promyelocytic Leukaemia seen in the University Hospital, Kuala Lumpur in the past year with special emphasis on morphology and laboratory findings.

MATERIALS AND METHODS

Seven cases of APL were diagnosed between September 1981 and July 1982 and accounted for about a third of the cases of Acute Myeloid Leukaemia presenting during that period.

Peripheral blood and bone marrow films stained by the May-Grunwald Giemsa stain were examined in all cases before therapy was started. Cytochemical studies included stains for myeloperoxidase, the esterases and the periodic acid schiff (PAS). Trepine biopsy was performed in only one case and none had chromosomal analysis. Three of the seven cases were studied by electron microscopy. All patients had coagulation studies to exclude disseminated intravascular coagulation prior to and during chemotherapy.

RESULTS

As shown in Table 1, the ages of the patients ranged from 6 years to 51 years with a mean of 21 years. Three of the seven patients were below the age of ten years. Only two patients were female.

Laboratory findings

Six patients presented with pancytopenia. The haemoglobin levels varied from 2.8 gms/dl to 9.7 gms/dl and the platelet counts from 8,000/cmm to 69,000/cmm.

A wide variety of morphological changes were exhibited by the erythrocytes, the commonest being the presence of macro-ovaiocytes. The total white cell count was 7,600/cmm in one patient and less than 3,800/cmm in all the rest. Abnormal leucocytes were seen in the peripheral blood of all cases and represented from 15 to 85% of the differential count. In all the six cases presenting with pancytopenia, the majority of abnormal cells were typical hypergranular promyelocytes. The colour of the cytoplasmic granules varied from purple to red. The nucleus was often bilobed or reniform and nucleoli were frequently obscured by the granules. Cells with similar nuclear characteristics but scanty granulation were also present but represented only a small proportion of the abnormal cells. Occa-

* Lecturer, Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur (Address for reprint requests).

** Associate Professor, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur.

TABLE 1
HAEMATOLOGICAL DATA AT THE TIME OF DIAGNOSIS OF
ACUTE PROMYELOCYTIC LEUKAEMIA

Patient ^s	Age	Sex	BLOOD				MARROW		COAGULATION				
			HB gms/dl	WCC x10 ³ /ccm	Plat. x10 ³ /ccm	Abnormal Cells%	Blasts	Promyel.	PT	APTT	TT	Fibri- nogen	FDP
1	7	M	2.8	1.5	38	20	0	76	N	N	N	N	N
2	34	M	5.3	7.6	11	87	0	91	+	ND	ND	+	+
3	9	M	6.2	1.2	18	15	4	92	+	N	N	N	N
4	19	F	5.0	2.8	22	46	0	85	N	+	+	+	+
5	51	M	5.3	3.8	8	85	8	81	N	N	N	N	+
6	29	M	9.7	1.5	69	21	7	54	N	N	N	+	+
7	6	F	9.7	3.8	25	64	3	92	+	N	N	N	+

N = Normal
 + = Abnormal
 ND = Not done
 PT = Prothrombin Time
 APTT = Activated Partial Thromboplastin Time
 TT = Thrombin Time
 FDP = Fibrinogen/fibrin degradation products

sional small promyelocytes with dark blue cytoplasm (the basophilic variant) were seen in two cases but were not conspicuous. The bone marrow was hypercellular in all cases and showed proliferation of abnormal promyelocytes with depression of normal haemopoiesis (Fig. 1). Cells containing multiple Auer rods were numerous. Erythropoiesis was megaloblastic in all cases.

One patient showed morphological features consistent with the microgranular variant of Acute Promyelocytic Leukaemia. The total leucocyte count was 7,600/cmm and abnormal cells accounted for about 85% of the differential count. These cells had only very occasional granules in the cytoplasm and the nuclei had distinct monocytoid features. (Fig. 2). The initial impression was an Acute Myelomonocytic Leukaemia (M4), but careful examination revealed the presence of a few typical hypergranular promyelocytes some of which contained multiple Auer rods. The appearance of the bone marrow was not conspicuously different from the other six cases although there was a higher proportion of the microgranular cells.

Cytochemistry

In all cases, the abnormal cells stained strongly positive for myeloperoxidase and chloracetate esterase. They were also stained with PAS but the pattern of staining varied from fine granules with a diffuse background to very strongly positive large blobs in the cytoplasm.

Electron Microscopy

Bone marrow from three patients was studied by electron microscopy. The typical hypergranular promyelocyte contained large, dense cytoplasmic granules in abundance, obscuring other details. The nuclei were rounded or lobulated and nucleoli were demonstrated in some cells. There was some peripheral condensation of chromatin in most cells. Auer rods, single or multiple were seen frequently. In the microgranular variant, the granules were small and sparse, and abundant, dilated rough endoplasmic reticulum (RER) was prominent (Figs 3 & 4).

Coagulation Studies

All patients were investigated for disseminated intravascular coagulation (DIC) before treatment. Various degrees of clotting abnormalities were noted in all four adults and one child, while two children showed no evidence of DIC. In all cases, marked abnormalities of coagulation were demonstrated after institution of cytotoxic therapy. In the majority, the prothrombin time was affected more often than the activated partial thromboplastin time (Table 1).

DISCUSSION

Classical Acute Promyelocytic Leukaemia or M3 is easily diagnosed by the presence of abnormal cells with coarse granules. Most patients are leucopenic at presentation and hence these cells are not conspicuous in the blood smear. Cells with multiple Auer rods or faggot cells are always plentiful in the bone marrow and sometimes in the peripheral blood.

The association of this condition with a haemorrhagic state has been recognised as far back as 1964⁶ and it is now widely accepted that the bleeding tendency is the result of disseminated intravascular coagulation. Gralnick² in 1973 studied the abnormal cells in APL and found the granules to contain both tissue thromboplastin as well as fibrinolytic activity but the former was present in excess of the latter. This resulted in the tendency to intravascular clotting in APL, especially at the time of chemotherapy. In spite of controversial reports, most authors advocate additional therapeutic measures such as prophylactic heparin therapy⁷ and vigorous support with platelets and plasma fractions. Cytogenetic studies of patients with leukaemia have revealed an abnormality which appears to be specific for APL which is a translocation $t(15q+17q-)$.³

Recently, morphologically atypical cases have been described where the leukaemic cells exhibit a monocyte-like appearance. Golomb⁴ studied three such cases in detail and found that although granules were not readily visible under light microscopy, transmission electron microscopy (TEM) showed their presence in great numbers. Furthermore, all three patients had laboratory evidence of disseminated intravascular coagulation and chromosomal abnormalities involving a 15:17 rearrangement. Although light microscopy suggested a diagnosis of Acute Myeloid Leukaemia with partial differentiation (M2), Golomb postulated that they were actually variants of Acute Promyelocytic Leukaemia or M3. In 1980, the French-American-British Co-operative Group proposed⁸ the inclusion of this subgroup as an M3 variant or microgranular variant of APL.

The most recent and largest series of 39 cases is by McKenna *et al* in 1981. 21% of cases were of the microgranular type and apart from the morphological differences, it was found that the median blood leucocyte count was significantly higher in the M3 variant group and the median duration of clinical remission was shorter. Another variant of the microgranular promyelocyte was identified which was small, showed unusual nuclear lobulation and deeply basophilic cytoplasm. There was no difference in the incidence of DIC or the specific karyotype. TEM revealed numerous small granules

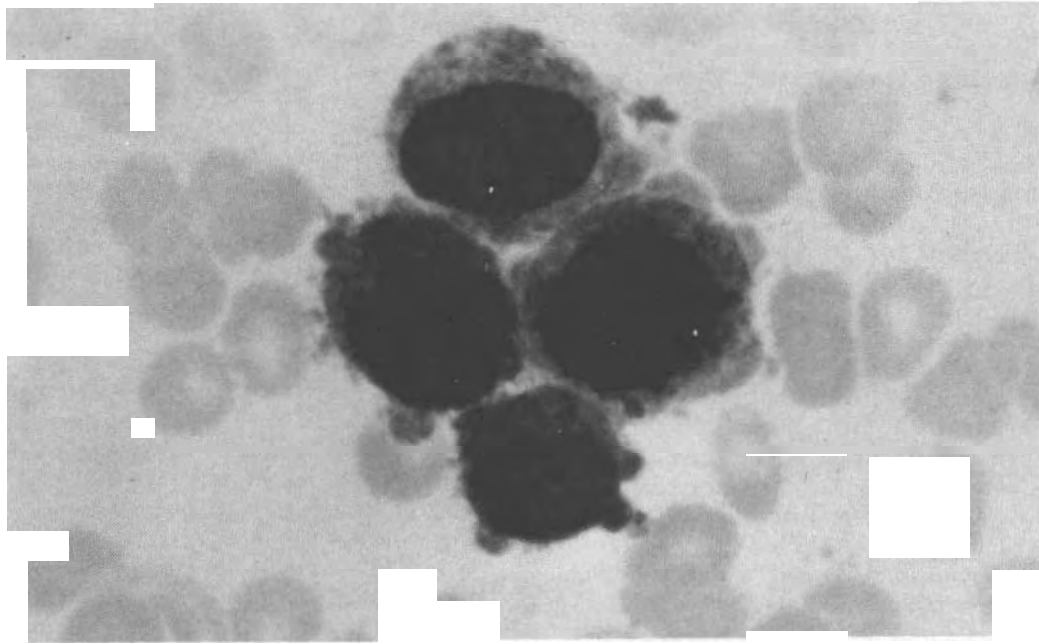


Fig. 1 : Bone marrow smears from a patient with APL. Hypergranular promyelocytes, some with cytoplasmic projections are illustrated. May-Grunwald-Giemsa stain X 1000.

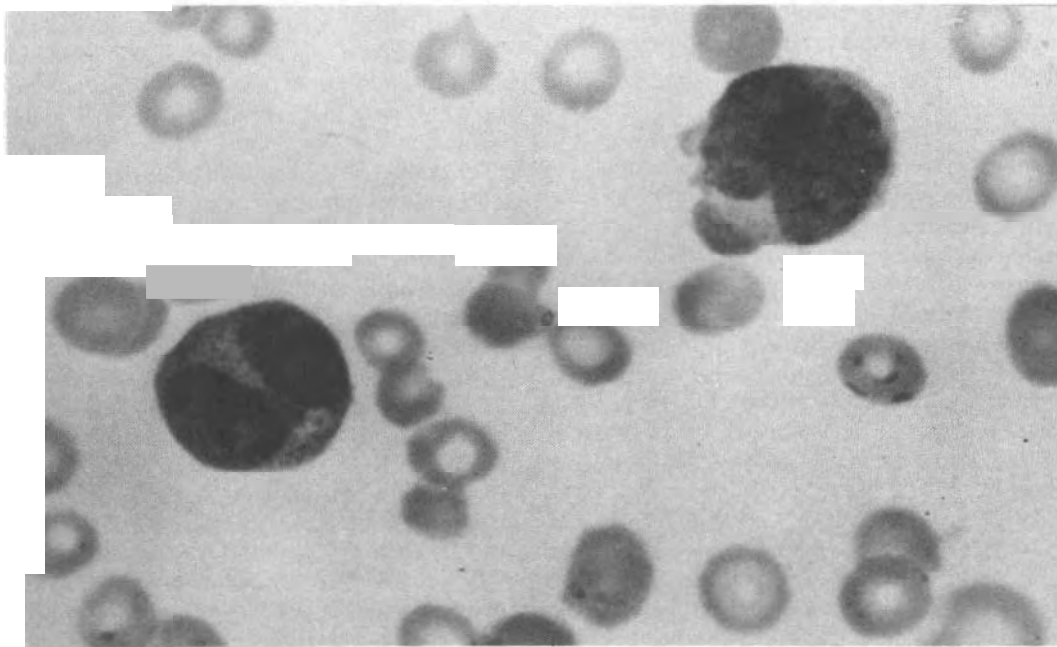


Fig. 2 : Peripheral blood smear from the patient with microgranular APL. Two abnormal cells with monocytoid nuclei and sparse granulation are seen. May-Grunwald-Giemsa stain X 1000.

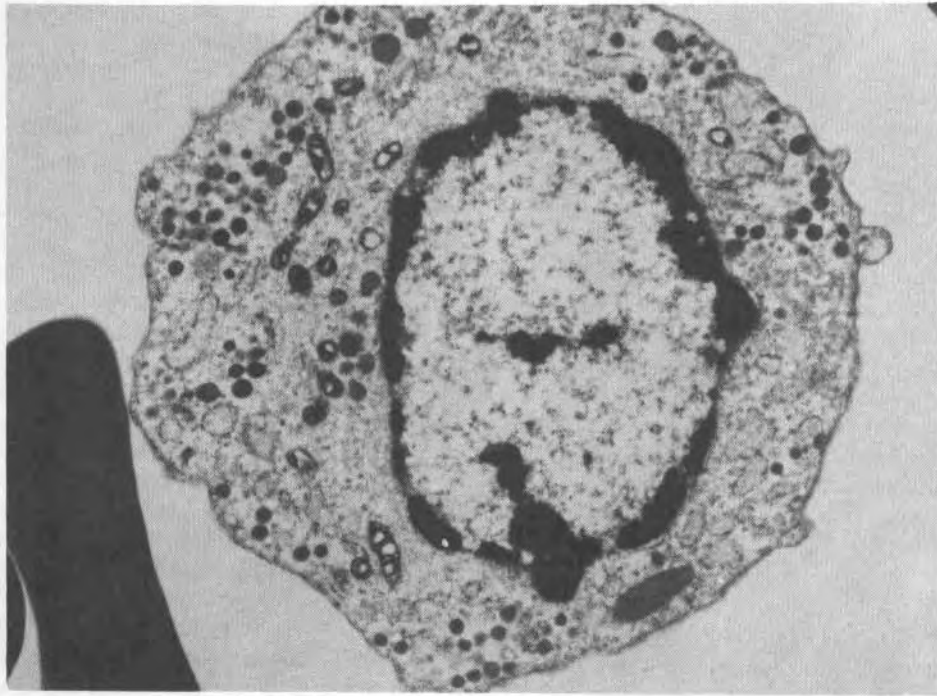


Fig. 3 : Electron micrograph of a leukaemic cell of microgranular APL showing sparse, fine granules and peripheral condensation of nuclear chromatin. Note the single Auer rod. Uranyl acetate-lead citrate stain X 14,000.

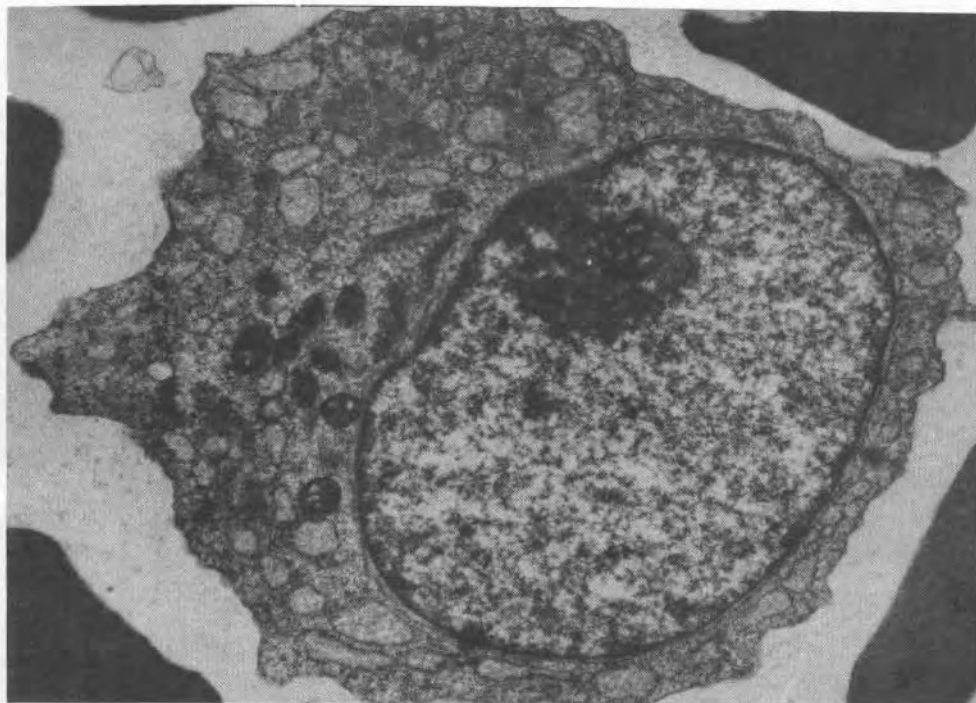


Fig. 4 : Electron micrograph of a leukaemic blast from a case of microgranular APL showing dilated rough endoplasmic reticulum and parts of 3 Auer rods. Uranyl acetate-lead citrate stain X 14,000.

and dilated, cistern-like rough endoplasmic reticulum.

In our small series of seven cases seen over a period of ten months, only one case was classified as microgranular APL. This patient did have a normal leucocyte count in contrast to the leucopenia demonstrated by the others. He had signs of intracranial haemorrhage at the time of admission and died before commencement of chemotherapy. Laboratory tests showed evidence of DIC.

It is important, therefore, not to rely on a single morphologic feature — the classical hypergranular promyelocyte to diagnose APL. Accurate identification is necessary because of the need for special therapeutic measures. Additional features such as multiple Auer rods and nuclear changes should be looked for. Doubtful cases should have coagulation studies before treatment and electron microscopy and cytogenetics may be used to verify the diagnosis.

REFERENCES

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. *Br J Haematol* 1976; 33: 451-8.
2. Gralnick HR, Abrell E. Studies of the procoagulant and fibrinolytic activity of promyelocytes in acute promyelocytic leukaemia. *Br J Haematol* 1973; 24: 89-99.
3. Testa JR, Golomb HM, Rowley JD, Vardiman JW, Sweet DL Jr. Hypergranular promyelocytic leukaemia (APL): cytogenetic and ultrastructural specificity. *Blood* 1978; 52: 272-80.
4. Golomb HM, Rowley JD, Vardiman JW, Testa JR, Butler A. 'Microgranular' acute promyelocytic leukaemia: a distinct clinical, ultrastructural, and cytogenetic entity. *Blood* 1980; 55: 253-9.
5. McKenna RW, Parkin J, Bloomfield CD, Sundberg RD, Brunning RD. Acute promyelocytic leukaemia: a study of 39 cases with identification of a hyperbasophilic microgranular variant. *Br J Haematol* 1982; 50: 201-14.
6. Baker WG, Bang NU, Nachman RL, Raafat R, Horowitz HI. Hypofibrinogenemic haemorrhage in acute myelogenous leukaemia treated with heparin. With autopsy findings of widespread intravascular clotting. *Ann Intern Med* 1964; 61: 116-23.
7. Drapkin RL, Gee TS, Dowling MD, et al. Prophylactic heparin therapy in acute promyelocytic leukaemia. *Cancer* 1978; 41: 2484-90.
8. Bennett JM, Catovsky D, Daniel MT, et al. A variant form of hypergranular promyelocytic leukaemia (M3). *Br J Haematol* 1980; 44: 169-70.