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Original article:

Study of diagnostic features of bone marrow in aplastic anaemia

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Abstract

Background: Aplastic anaemia is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin. The incidence of aplastic anemia is subjected to wide variation. Most cases are acquired and immune-mediated but there are also inherited forms. The study was conducted to assess the morphological changes in aplastic anemia in order to assess the extent of dyserythropoiesis and to evaluate the use of bone marrow cytology.

Materials and Methods:A prospect study had been conducted for a period of two years among all age group. Initially complete blood count followed by bone marrow examination was done for diagnosis.

Result : Bone marrow preparations were examined from 50 patients. The degree of cellularity varied greatly . In the severely hypoplastic marrows lymphoid cells were predominant. There was no correlation between marrow lymphoid cell content and blood lymphocyte count but there was an inverse relationship between blood lymphocyte count and marrow erythroblasts and a close direct relationship between the blood neutrophil count and marrow myeloid cell content. In all cases a proportion of the erythroblasts showed morphological abnormalities. These included especially megaloblastic changes and asynchrony of nuclear-cytoplasmic maturation. There were also binucleated cells, internuclear chromatin bridges, intercellular cytoplasmic connexions, nuclear degenerative changes, namely, blurred outlines, irregular shapes, budding and fragmentation, and atypical mitotic figures.

Conclusion : These appearances illustrate the extent to which a qualitative defect of erythropoiesis occurs as part of the haematological pattern in aplastic anemia, and in some cases dominates the bone marrow picture. Similar cytological features were found in all cases.

Keywords: Aplastic anemia, pancytopenia, dyserythropoiesis, hypocellular marrow

Introduction

The term aplastic anemia (AA) (synonyms: panmyelopathy, panmyelophthisis) comprises a group of pathogenetically heterogeneous bone marrow failures. They are characterized by a tricytopenia (anemia, granulocytopenia, thrombocytopenia) which arises from hemopoietic failure due to hypoplasia or aplasia of the bone marrow [1].

The incidence of acquired aplastic anaemia in Europe and North America is around 2 per million population per year (Issaragrisil et al, 2006; Montane´ et al, 2008) (2,3). The incidence is 2–3 times higher in East Asia. There is a biphasic age distribution with peaks from 10 to 25 years and >60 years. There is no significant difference in incidence between males and females (Heimpel, 2000) (4). Congenital aplastic anaemia is very rare, the commonest type being Fanconi anaemia, which is inherited as an autosomal recessive disorder in most cases.

Haematopoiesis is affected in two ways in aplastic anaemia. In addition to a quantitative decrease in haematopoeitic cells, there are qualitative alterations. In the erythroid cells it results in the production of a proportion of defective cells; these are characterized by morphological abnormalities such as poikilocytosis
and fragmentation, decreased survival in vivo. These features indicate that dyserythropoiesis is a characteristic of the aplastic anaemias, albeit usually overshadowed by the hypoplasia.

Objective - The purpose of this study was to review bone marrow appearances in aplastic anaemia in order to assess the extent of dyserythropoiesis and to evaluate the use of bone marrow cytology as a guide to diagnosis.

Method and material:

The present prospective study was undertaken for a period of 2 years, from July 2016 to Sept 2018, at Hematology Unit, Department of Pathology. Patients of all age group and both sexes were included.

A total of 50 bone marrow aspirates was examined. The patients had been diagnosed as suffering from aplastic anaemia on the results of peripheral blood counts and bone marrow aspiration and biopsies, and their clinical course. Patients with pure red cell anaemia have not been included.

In this study *'Jamshidi Needle'* had been used. Posterior iliac spine was the site of choice. The preparation was done according to the standard procedure [9,10,11] After air-drying the slides were fixed in methanol and stained with field stain. The films were examined by light microscopy

In 25 cases selected at random, the relative distribution of erythroid, myeloid, and lymphoid cells was estimated from a count of 500 nucleated cells in each marrow preparation; with markedly hypocellular preparations it was necessary to scan more than one smear of the same aspirate to reach the required count. The presence of cells other than those listed above was also noted: these included megakaryocytes, reticulum cells and connective tissue cells, phagocytes with and without cellular debris, mast cells, basophils, plasma cells, and monocytes.

DEGREE OF CELLULARITY

This was graded 1-6 as follows:

Grade 1

Although material was undoubtedly of marrow origin, as evidenced by the presence of isolated
reticulum cells, phagocytes, mast cells, and scattered haemopoeitic cells, there were no fragments
and no organized cellularity.

Grade 2

Fragments of fatty tissue containing phagocytes and reticulum cells with a small amount of haemopoietic
tissue in the fragments and in the trails.

Grade 3

Some haemopoietic tissue in trails and in the main body of the film.

Grade 4

Fragments present; normal cellularity within the fragments and in the trails.

Grade 5

Normal numbers of fragments with moderately increased cellularity.

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Grade 6

Hypercellular, often with abundant fragments.

MORPHOLOGY OF ERYTHROID CELLS

The erythroblasts were classified as follows: (a) normal early erythroblasts, i.e., up to polychromatic stage; (b) normal late erythroblasts, ie, haemoglobinized cells; (c) abnormal early erythroblasts; (d) abnormal late erythroblasts. The morphological criteria for abnormality were as follows: asynchrony of maturation between nucleus and cytoplasm, mitotic abnormalities, binuclearity or multinuclearity, nuclear lobulation, budding, fragmentation, internuclear bridging, and megaloblastosis. These abnormalities are characteristic of dyserythropoiesis (9). Cytoplasmic vacuolation, basophilic stippling of the cytoplasm, and cytoplasmic connexions between erythroid cells were also taken to indicate dyserythropoiesis. For scoring the percentage of abnormal (dyserythropoietic) cells, any erythroblast showing one or more of the above features was classified as being abnormal. Five hundred erythroblasts were examined in each case.

Results and Observations

Marrow cellularity :

The cellularity of the aspirated marrows varied considerably. In 24 bone marrows aspirates the marrow was hypocellular (fig 1) whereas in 26 marrows it was normal or even Hypercellular . In several of the cases the cellularity of a second aspirate differed from that of the first; such variability might occur within a small area of marrow (fig 1). The peripheral blood cytopenia usually but not always paralleled the degree of bone marrow hypoplasia .

Marrow cell populations :

The myeloid :erythroid (M :E) ratio ranged from 0.5 :1 to 10:1 (normal 2-5:1).

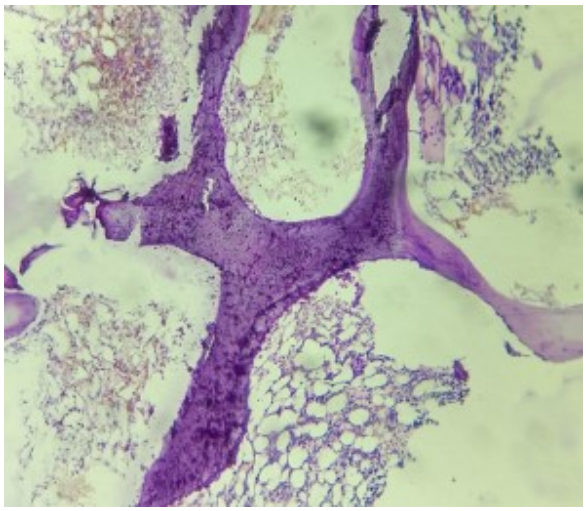

Figure 1 a

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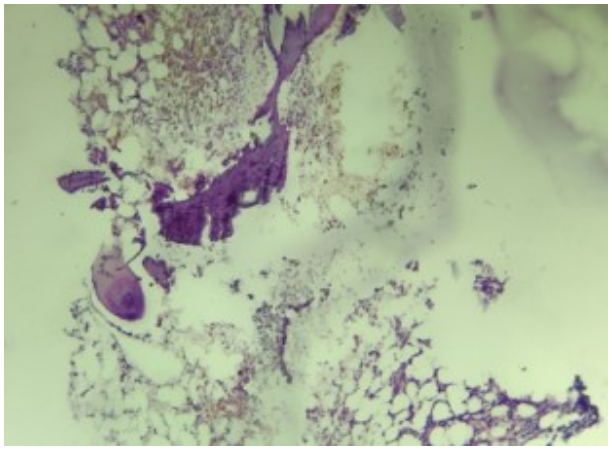

Figure 1 B

1 A & B Sections of bone marrow biopsy from a case of aplastic anaemia. H. & E. stain; x 10

The predominant cell type varied between cases. In some there was a normal relative distribution. In many cases, however, especially in the more severe hypoplasias, lymphoid cells were predominant
(figure 1 and 2) and there was an inverse correlation between lymphoid and erythroid cells (fig 3).
As a rule there was a normal relative distribution of myeloid cells.

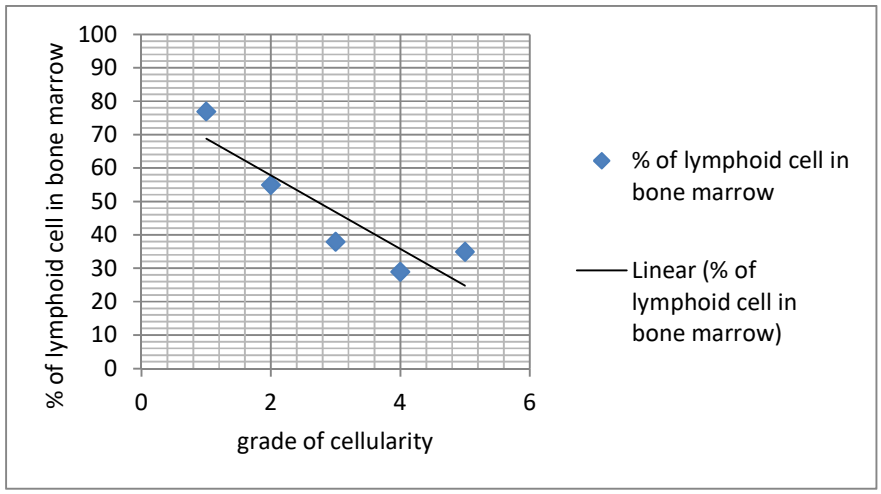

Figure 2

Fig. 2 Occurrence of lymphoid cells in bone marrow related to cellularity. The grades of marrow cellularity are described in the text; grade I is the most markedly hypocellular and grade 6 is hypercellular.

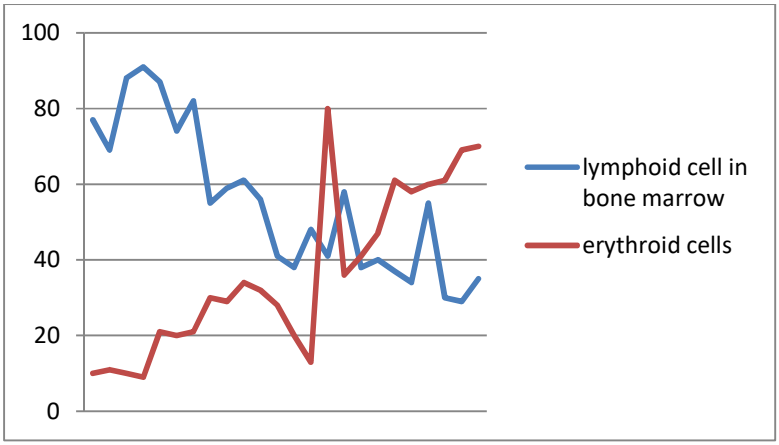

Figure 3

Fig 3 Proportional relationship of lymphoid cells to erythroblasts in the bone marrow in aplastic anemia. All grades of marrow cellularity are included.

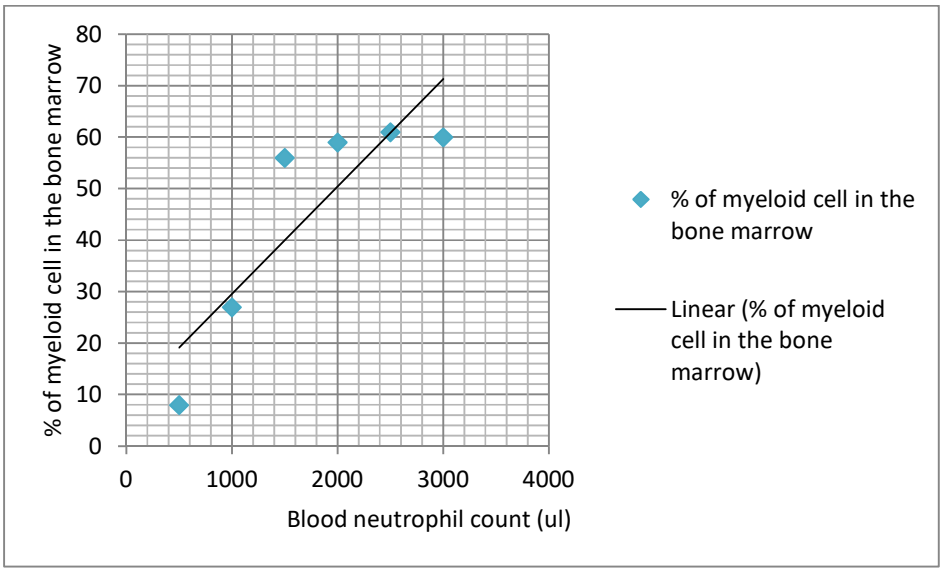

Fig 4 Relationship of bone-marrow myeloid cells to peripheral blood neutropenia in aplastic anaemia.

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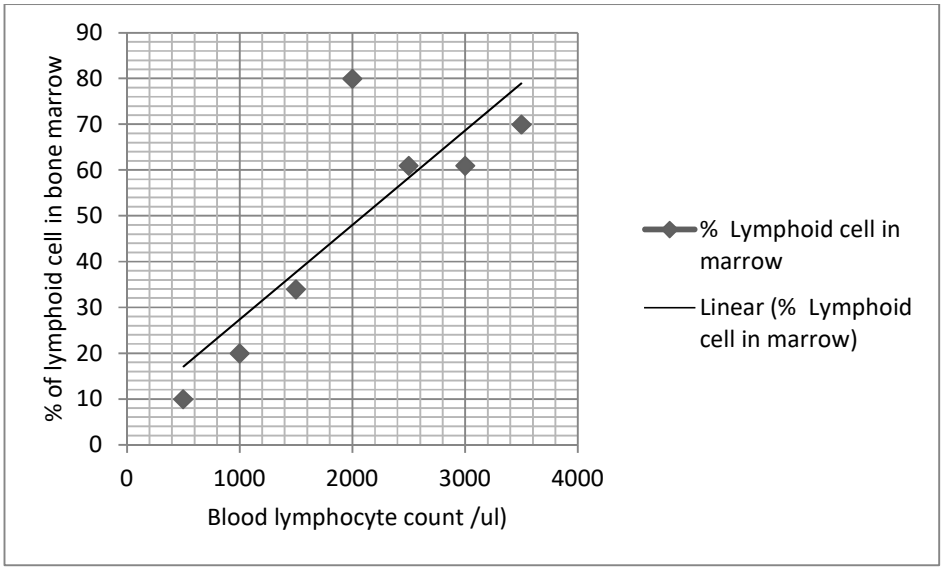

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Figure 5

Fig 5 Relationship of bone-marrow lymphoid cells to blood lymphocyte count in aplastic anaemia.

The relationship of cell type predominating in the bone marrow to the peripheral blood leucocyte count was studied. Not unexpectedly, a high relative myeloid cell content in the marrow was associated with a less severe degree of neutropenia and the correlation was remarkably close (fig 4).

On the other hand, there was no correlation between bone marrow lymphoid cells and blood lymphocyte count (fig 5).

'Reticulum cell'

Under this heading are included phagocytes and connective tissue cells. These were present in all hypoplastic marrows, frequently in association with mast cells (fig 7), and they were seen in both acquired and congenital aplastic anaemia. Many of the phagocytes contained cellular debris, especially in the more cellular marrows, and phagocytes containing numerous vacuoles were also observed (fig 8).

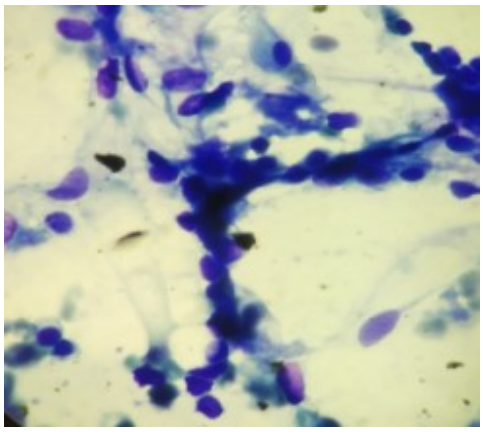

Fig 7 Photomicrograph of hypoplastic marrow showing reticulum cells and mast cell X 100.

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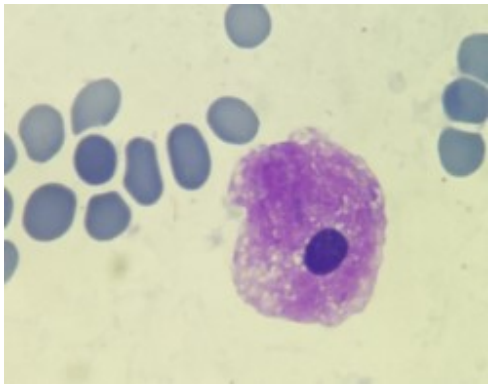

Fig 8 Photomicrographs of bone marrow, from patient with aplastic anaemia showing presence of macrophage

Megakaryocytes

Megakaryocytes were not generally to be found in hypocellular marrows of grades 1 and 2. Nonetheless, platelets were frequently seen in these smears, an indication that megakaryocytes were not completely absent. Scattered megakaryocytes were found in relatively cellular marrows .

Dyserythropoiesis

Dyserythropoiesis was present in every case. In a few only 5 % of the erythroid cells were abnormal, but in the majority of cases more than half of the erythroid cells. Dyserythropoiesis occurred with all grades of hypocellularity and at any stage of cell development. The following features, some of which are illustrated in fig 10 (A-M), were especially noteworthy. Megaloblastosis and asynchrony of maturation of nucleus and cytoplasm (A,B) were the most frequent abnormalities. They occurred in every case and in some the majority of the erythroblasts were megaloblastic. Binucleated erythroid cells (C) were present in about half of the cases. In a few they were an outstanding feature, but in the majority of cases only 1-5 % of the normoblasts had double nuclei. Both early and late normoblasts were affected; in some of the binucleated cells asynchronous development of the paired nuclei was a striking feature.

Atypical mitotic figures (D,E) were found in both early and late erythroid cells in almost all cases.

Cytoplasmic connexions between erythroid cells of varying degrees of maturity (E,F,G,H) were found in up to 4 % of the erythroid cells both early and late. Occasionally there was a striking degree of asynchrony between cells thus joined. This abnormality was seen in 80% of the marrows.

Chromatin bridges between nuclei (I) also occurred but they were much less frequent than cytoplasmic connexions. In some preparations, extrusion of the nucleus was seen in relatively immature erythroblasts . Bare nuclei and cytoplasmic masses (often basophilic) were also found.

In all marrows a small percentage of normoblasts showed degenerative changes in their nuclei (J , K) -blurred nuclear outlines, irregular shapes, budding, and fragmentation. In some cases vacuolations (M) could be seen around the nucleus and occasionally between the nuclei of a binucleate erythroblast. More generalized cytoplasmic vacuolation was rare. Basophilic stippling (L) were seen in 1-2% of the erythroblasts

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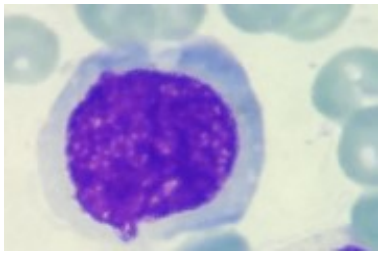


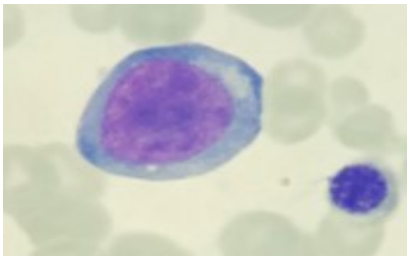
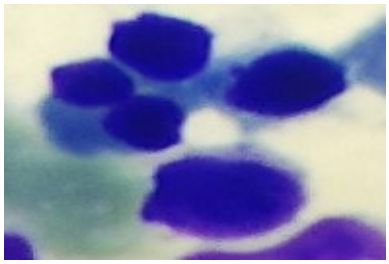
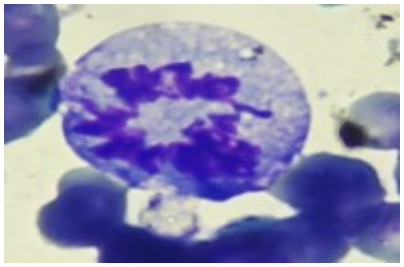
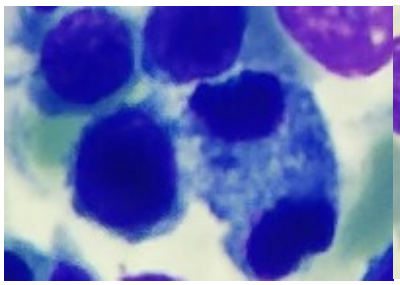


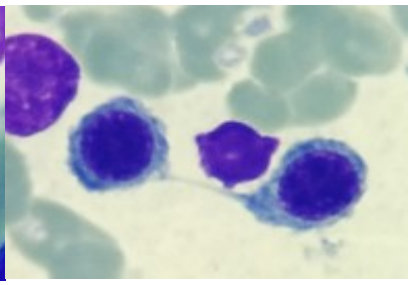
Fig 10 A B





C D





E F

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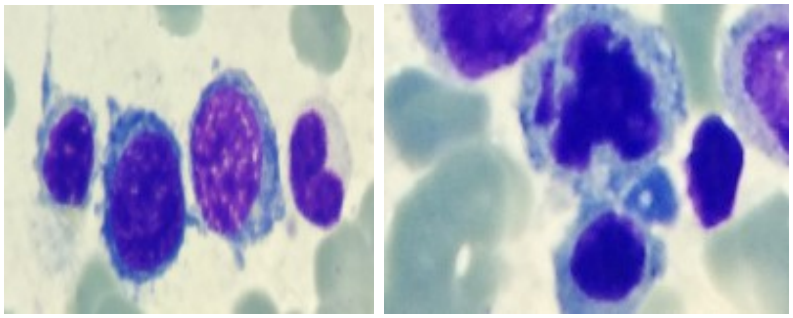
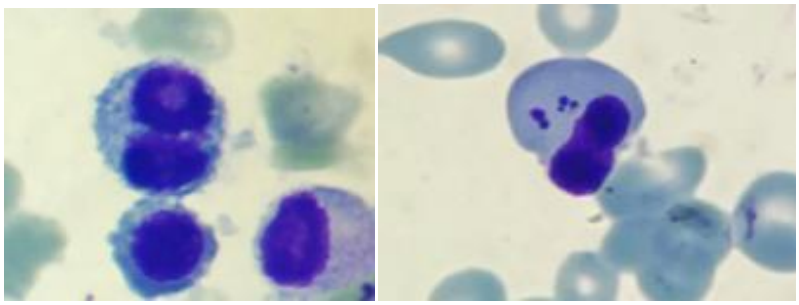


J H



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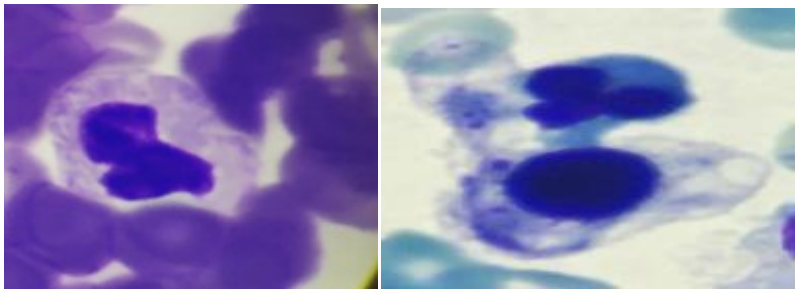
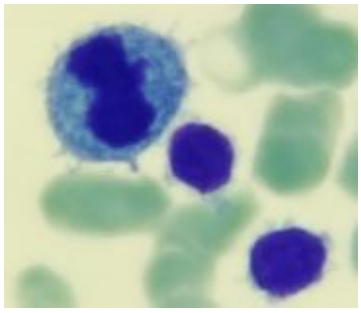


K L



M

Fig 10 (A – M) Photomicrograph of bone marrow from patient with aplastic anaemia showing various features of dyserythropoiesis

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Discussion

Whereas aplastic anemia is characterized by a quantitative deficiency in cell production within the bone marrow, there is also frequently an associated qualitative defect in erythropoiesis . The present study has shown the extent to which morphological abnormalities occur. The term dyserythropoiesis expresses both the kinetic and morphological aspects of abnormal erythropoiesis (9). Kinetically, there is ineffective erythropoiesis with intramedullary destruction of erythroblasts.

Dyserythropoiesis of greater or lesser severity occurs in a wide range of diseases. Several types of congenital dyserythropoietic anemia have been described and dyserythropoiesis is also evident in myelosclerosis, leukaemia, megaloblastic anaemias, iron deficiency anaemia, and in defects of iron utilization as found in acquired sideroblastic anaemias and infections. From the static pictures of cells as seen in a marrow aspirate, it is difficult to deduce the dynamic processes involved in abnormal cytopoiesis; furthermore many of the morphological manifestations of dyserythropoiesis may occur as the result of a disturbance at any stage of erythropoiesis. In aplastic anemia, some of the earliest recognizable erythroblasts show megaloblastic changes, indicating interference with DNA synthesis. Disturbances of the mechanism which regulates the cell cycle and normal mitosis results in multiple nuclei. Asynchronous maturation of each nucleus in a cell and premature extrusion from inadequately hemoglobinized cells are also consequences of defective cell differentiation. Other manifestations, such as nuclear budding and fragmentation, as well as cytoplasmic vacuolation, are probably degenerative.

The pressure exerted on cells during extraction and smearing may lead to distortion, with stretching or rupture of intercellular connexions and displacement or fragmentation of intracellular structures. However, disturbance in protein synthesis may cause the cell to be more fragile and thus more easily damaged by these mechanical actions; it may also lead to a defective membrane which results in poikilocytosis and cell fragmentation

The variability of marrow cellularity in aplastic anaemia has been previously noted (9). This is likely to result in unrepresentative sampling and the possibility that evaluation of a single bone marrow aspiration might lead to an erroneous diagnosis (fig 1). Furthermore, the appearance in aspirated material may differ from trephine sections; this may be due to residual marrow being less readily dislodged by aspiration in aplastic anaemia. The relative proportion of different cell lines within the bone marrow aspirate varies greatly from case to case. Within each of the cell lines, cells of all stages of maturation were present, although in the myeloid series there was a relative preponderance of early cells.

In our study, in all the cases there is no blasts with normal or reduced myelopoiesis Increased blasts are not seen in aplastic anaemia, and their presence either indicates a hypocellular MDS or evolution to leukaemia . (12,15)

Hypocellular MDS/acute myeloid leukaemia (AML) can sometimes be difficult to distinguish from aplastic anaemia. The following features of MDS are not found in aplastic anaemia: dysplastic cells of the granulocytic and megakaryocytic lineages, blasts in the blood or marrow. (13,14,15)

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Conclusion

The study shows aplastic anemia is a common hematological abnormality among peripheral pancytopenia. On morphological study dyserythropoiesis is common finding in all the cases. Bone marrow aspiration with biopsy is confirmatory diagnostic tool for aplastic anemia.

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