Nigerian Journal of Paediatrics 2003; **30**(2)54-59

Differential Cell Count of Bone Marrow Aspirates in Steady-state Sickle Cell Anaemia Patients

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Summary

Akinsulie AO, Akanmu AS, Esan OA. Differential Cell Count of Bone Marrow Aspirates in Steady-state Sickle Cell Anaemia Patients. *Nigerian Journal of Paediatrics* **2003; 30:54**

Background: Megaloblastic arrest of the bone marrow is one of the causes of anaemic crises in patients with sickle cell anaemia. The diagnosis of this condition will require that the reference level of megalobalastosis during steady state be properly documented.

Objectives: To document a reference differential cell count in the bone marrow aspirates of patients with sickle cell anaemia and determine the level of megaloblastosis that can be regarded as indicating megaloblastic crisis in such patients.

Design: Systematic counting of haematopoietic cells in the bone marrow of children with confirmed Hb phenotype SS who are in steady state.

Subjects and Methods: The subjects were 11 of 68 children with sickle cell anaemia attending the paediatric outpatient clinic at the Lagos University Teaching Hospital, Lagos, in respect of whom parental consent for bone marrow aspiration was obtained. About 4.5 ml of blood was obtained from the antecubital vein of each child, for full blood count. Bone marrow was aspirated from the posterior superior iliac spine. Slides were stained with MayGrünwald-Giemsa stain. Proportions of erythroid, myeloid, lymphoid and megakaryocytic cells out of 250 nucleated bone marrow cells were determined.

Results: Steady state mean packed cell volume (PCV) was 0.2 ± 0.017 L/L. The mean reticulocyte count was 5.9 percent (95% CI, 5.3 – 7.0%) and the mean cell volume was 91.8 ± 2.7 fl. Erythroid precursors constituted 34.5 percent of the total nucleated bone marrow cells (NBMC). Of these, polychromatic and orthochromatic erythroid blasts predominated, constituting 49 and 36 percent respectively, of total erythroid precursors. Polychromatic and orthochromatic megaloblasts constituted 16.5 percent (95% CI, 7-25%) of total NBMC or 47.8 percent of erythroid precursors. The myeloid erythroid ratio was 1:1. The reference range of megaloblasts was 8-26 percent of the NBMC.

Conclusion: Patients with sickle cell anaemia in steady-state may show megaloblastic bone marrow changes even with routine folate supplements. Megaloblastic crisis should not be diagnosed until megaloblasts are in excess of 26 percent of the total NBMC.

Introduction

THE normal bone marrow tissue is 3.4 – 5.9 percent of total body weight in the adult, a value approximately equivalent to the weight of the liver.[1] The red marrow responsible for the production of haemopoietic cells weighs one kg. This red tissue however, has a tremendous capacity for expansion in disease states.[2] The mechanisms of marrow expansion are: (a)increased parenchymal cellularity with loss of fat in areas of normal haematopoiesis, (b) expansion of marrow into previously inactive marrow cavities, and (c) shortening of cellular maturation time. Sickle cell anaemia (SCA) is a chronic haemolytic state and thus, it is expected that bone marrow expansion resulting in erythroid hyperplasia should be a common finding in marrow aspirates of these patients. It is unclear if this is truly the case. A common cause of severe anaemic crises in these patients is acute megaloblastic arrest due to folate deficiency. This is occasioned by (i) dietary deficiencies

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as a result of interruption of food intake often consequent on intercurrent illnesses[3] such as diarrhoeal diseases,[4] (ii) period of rapid growth,[5] and (iii) the additional haemolytic load from malaria in malaria-endemic areas.[6] Diagnosis of megaloblastic arrest in a sickle cell anaemia patient will require that the reference level of megaloblastosis during steady chronic haemolytic state be properly documented. As such, bone marrow aspirate studies will be necessary for a number of reasons: (a) to document the normal findings in the bone marrow of steady-state SCA patients, (b) to study marrow aspirate cellularity of these patients. This is because the usual sluggishness of blood flow in the bone marrow sinusoidal vessels subject the HbS containing red cells to anoxia, sickling and bone marrow infarctions. Such recurrent infarctions may impair the expected expansive response of bone marrow to chronic anaemia in these patients, and (c) to document reference levels of megaloblastosis during a steady chronic haemolytic state. There is need for this documentation because SCA as a chronic haemolytic state is a state of increased folate demand and the bone marrow may show megaloblastic changes.[7]

Except for infarctive bone changes,[8,9] reports of steady state bone marrow aspirate findings in SCA patients is scanty in the literature. The present communication is aimed at shedding more light in this area.

Subjects and Methods

Steady state SCA patients were recruited into the study. All the patients were attending the paediatric outpatient clinic at the Lagos University Teaching Hospital. They were all on routine folic acid 5mg daily supplements. A patient was regarded as being in steady state if he/she had a PCV greater than 0.15 and had suffered no crises in the three months immediately preceding the study. The exclusion criteria were (a) any form of sickle cell crisis, (b) diarrhoeal disease and (c) blood transfusion during the three months immediately preceding the study.

Blood specimen was obtained from an antecubital vein between 9 and 11 a.m. About 4.5 ml of the blood was placed in an EDTA bottle for determination of PCV(L/L), reticulocyte count (percent), mean cell volume(fl), platelet count(x 10^9/L), total white cell count (x 10^9/L) and its differential according to standard methods as described by Dacie and Lewis.[10] Bone marrow aspirate was obtained from the posterior superior iliac spine. Both the peripheral film and the marrow slides were prepared using May-Grünwald Giemsa stain. The differential counts of the peripheral blood and bone marrow film were determined by counting 250 cells by two of the authors. Small sample size statistics was employed to define a reference range (RR) for megaloblasts in the marrow according to the

Table I

Peripheral Blood Counts in 11 Steady State Sickle Cell Anaemia Patients

Haematologic Parameter	*Number*	Σx	*Mean*	*SD*	*Range*	*Reference Range for SCA patients*
PCV L/L	11	217	0.20	0.017	0.18 - 0.23	0.19 - 0.21
Total WBC x 10^9/L	11	981	8.9	2.1	6.5 - 13.5	7-10.8
Differential Leucocyte Count (%)						
Neutrophils	9	405	45	18.1	24-69	26.9 – 63.1
Lymphocytes	9	435	48.3	15.7	25-69	32.6 – 64
Eosinophils	9	56	6.2	6.85	0-23	0 – 13.05
Monocytes	9	4	0.44	1.0	0-3	0 – 1.4
Basophils	9	0	0	0	0	0
Platelet Count x 10^9/L	6	1905	317.5	78.6	235-440	221.3– 413.7
Reticulocytes (%)	11	65	5.9	1.9	3-9	4.2 – 7.6
MCV (fl)	11	1010	91.8	2.7	88-96	89.4 – 94.2

Σx = *The sum of means*

* Two slides were poorly prepared and not suitable for differential count.

** Platelet count not available for 5 patients.

formula: RR = x ± 3SEM, where x is the mean value and SEM is the standard error of mean.

Informed consent was obtained from the parents or guardians of the subjects. The study was approved by the Ethical Committee of the Lagos University Teaching Hospital.

Results

Of the 68 steady state SCA patients that were seen in the two-month study period, informed consent for bone marrow aspiration was obtained in respect of only 11. Of these, four were females and seven males. Their ages ranged from two to nine years with a mean of 6.7 years (SD ± 2.4).

Peripheral Blood Counts

The mean PCV was 0.2 ± 0.017(L/L) with a reference range (RR) of 0.19 – 0.21. The mean reticulocyte was 5.9 percent (95% CI of 5.3 – 7.0%). The mean cell volume varied between 89 and 94fl with a mean of 91.8 ± 2.7fl. The median platelet count was 310 x 10^9/L with a mean of 317.5 ± 78.6 x 10^9/L. Total white cell count varied between 6 x 10^9/L and 22.6 x 10^9/L with a median of 8.3 x 10^9/L; the mean was 8.9 x 10^9/L. Proportions of neutrophils (45 percent) and lymphocytes (48.3 percent) were not significantly different (SE = 7.98, P = 0.68).

Differential Erythroid Precursor Cells Counts

A total of 2,750 nucleated bone marrow cells (NBMC) were counted (250 cells from each of the 11 subjects); 948 (34.5 percent) of these were erythroid cells. The median value was 32.4 percent, standard error 3.29, and 95% CI, 27.9 – 41.1%. Of the total erythroid cells, polychromatic erythroid blasts predominated, constituting 49 percent of all erythroid cells or 16.9 percent of the total NBMC. Orthochromatic erythroid blasts constituted 12.4 percent out of the total NBMC or 36 percent of erythroid cells. Polychromatic and orthochromatic megaloblasts were also found frequently. The median megaloblast proportion was 14.8 percent. On the whole, 454 (47.8 percent) of the 948 erythroid cells were megaloblasts. This translates to 16.5 percent of total NBMC (Table II). There was an insignificant negative correlation between megaloblastosis and PCV (p=0.47), and an insignificant positive correlation between megaloblastosis and mean cell volume (p=0.377).

Myeloid Differential Cell Counts

The sum of neutrophilic (902) and eosinophilic (61) cells approximated the total erythroid cells (948) giving a myeloid : erythroid ratio (M:E) of 1:1. In one subject, M:E was 1:2 (reversed) and 3:1 (normal) in another. In the remaining nine subjects, it varied between 1.3:1 and 1:1.4 (reduced).

The total neutrophilic series constituted 32.8 percent of the total NBMC with SE of 1.2 (95% CI of 29.4 - 36.2%). All the developmental stages namely,

Table II

Differential Counts of Erythroid Precursors in Marrow Aspirates of 11 Patients with Sickle cell anaemia in Steady State

Cell Types	*Sum total of percentages*	*Mean (%)*	*SD*	*Range*	*Reference Range x ± 3SEM*
Total erythroid	387.7	34.5	10.92	12.4 – 48.8	24.5 – 44.4
Pronormoblasts	3.6	0.38	0.39	0.0 – 0.8	0.03 – 0.73
Basophilic normoblast	53.2	4.84	8.5	0 – 9.6	1.6 – 81
Polychromatic blasts (Normoblasts + Megaloblasts)	186	16.91	8.97	5.2 – 30.8	8.8 – 25.0
Orthochromatic blasts (Normoblasts + Megaloblasts)	136.4	12.4	5.75	6 – 22.4	7.2 – 17.6
Megaloblasts (Polychromatic and Orthochromatic)	181.6	16.9	9.9	2 – 36	7.9 – 25.9

X = mean
ME ratio = 1:1

Table III

Differential Count of Myeloid, Lymphoid and Megakaryocytic Precursors in Marrow Aspirates of 11 Patients with SCA in Steady State

Cell Types	*Sum total of percentages*	*Mean (%)*	*SD*	*Range*	*Reference Range* $x \pm 3SEM$
Neutrophilic Series (Total)	360.8	32.8	5.7	23.2 – 41.2	27.6 – 38.90
Myeloblasts	23.2	2.1	0.78	1.2 – 3.6	1.4 – 2.8
Promyelocytes	16.8	1.53	0.59	1.2 – 2.8	1.0 – 2.1
Myelocytes	71.2	6.5	2.1	3.2 – 11.6	4.5 – 8.4
Metamyelocytes	62.8	5.7	2.3	3.2 – 10.0	3.6 – 7.8
Bands	85.2	7.8	2.0	4.8 – 11.2	5.9 – 9.6
Segmented neutrophils	101.2	9.2	3.4	4.4 – 15.6	6.2 – 12.3
Eosinophilic Series (Total)	24.4	2.2	1.8	0.8 – 4.4	0.6 – 3.9
Myelocytes	10.4	1.0	1.2	0.4 – 4	0.0 – 2.0
Metamyelocytes	2.8	0.25	0.5	0 – 1.2	0.0 – 0.70
Bands	1.2	0.1	0.3	0.0. – 0.8	0.0 – 0.35
Segmented eosinophils	10.0	0.9	1.0	0.4 – 3.6	0 – 1.80
Lymphocytes	312.4	28.4	7.8	20.4 – 40.8	21.3 – 35.5
Plasma Cells	10.4	1.0	1.1	0.0 – 4.0	0.0 – 2.0
Monocytes	4.0	0.36	0.4	0.0 – 1.2	0.0 – 0.7
Megakaryocytes	5.2	0.5	0.2	0.4 – 0.8	0.3 – 0.9

myeloblast, promyelocytes, myelocytes, metamyelocytes, bands and segmented neutrophils, were proportionately represented constituting 2.1, 1.5, 6.5, 5.7, 7.8 and 9.2 percent of the NBMC, respectively. Eosinophil proportions varied between 0.8 and 4.4 percent with a mean of 2.2 ± 1.8. Segmented and myelocyte eosinophils each constituted 41 and 43.6 percent respectively, of the eosinophil series or 0.91 and 0.96 percent of the NBMC (Table III).

Lymphocytes and other haematological cells in the marrow

The mean lymphocyte differential count was 28.4 ± 7.8 percent (Table III); the median was 22.4 percent. The plasma cells were scanty constituting 1.1 percent of the NBMC, with a median value of 0.4 percent. Monocyte macrophages were also rare. The mean value out of NBMC was 0.36 ± 0.42. In ten of the subjects, one megakaryocyte was seen out of the 250 NBMC. The last subject had two cells. The megakaryocytic differential count was 0.47 ± 0.16 (SE = 0.05; 95% CI = 0.37 - 0.56%).

Discussion

In this study, only 11 of the parents of the 68 SCA patients seen within the study period gave informed consent for bone marrow aspiration on their wards. The procedure was invasive and was not considered to contribute in anyway to the management of their children. This attitude may also explain why few reports are available in the literature on marrow findings in SCA patients. It may also explain the small sample size (n = 12) in bone marrow studies of healthy adults as reported by Wintrobe[1]. However, a study of the bone marrow picture in the steady state is needed to be able to properly interprete findings during periods of anaemic crisis. Chronic anaemia with haemoglobin level varying between five and 11g/dl is the usual finding in steady state SCA patients,[11] the higher values being commonly found in those aged above 10 years but below 40 years.[12] The PCV range of 0.18 – 0.23 noted in this study, is therefore consistent with expected findings in the study age group two to nine years.

The mean cell volume is said to be higher in SS disease than in AA genotype controls and that this

difference is noticeable from the eighth month of life.[12] The present value of 91.8 ± 2.7fl is within the upper range of adult values of 88 ± 7.9fl.[13] Some workers have described the anaemia in SCA as normocytic, normochromic in spite of the elevated reticulocyte count in these patients.[14] In comparison with patients with a similarly increased reticulocyte count, these patients may be considered to have a 'microcytic' anemia, presumably because the sickle mutation impairs the efficiency of production of hemoglobin.[14]

Elevated leucocyte count is a common finding in steady state SCA patients[15] and may be due in part, to a redistribution of neutrophils from the marginal to the circulating pool and does not necessarily indicate infection.[16] All the 11 subjects in this series including the patients with a count of 22.6 x 10^9/L were clinically well. Thrombocytosis is also a common finding in steady-state SCA patients[17] and is thought to be related to hyposplenism in this condition.[18]

The major alteration in the bone marrow in haemolytic anaemia is erythroid hyperplasia[19] and quantitative methods have been developed for determining the erythroid cell mass, but these methods are not practicable for routine clinical use.[19] A more practical assessment of erythroid hyperplasia is myeloid to erythroid precursor ratio (M:E ratio) which is often significantly reduced from about 2.3:1 to less than 1.5:1.[19] The finding of hypercellular bone marrow aspirares with a M:E ratio of 1:1 in this study is in keeping with the expected findings in haemolytic anaemias. In the healthy bone marrow smear, about 84 percent, and eight percent of the total erythroid cell are usually due to polychromatic and orthochromatic erythroblasts, respectively.[1] In this series, 48 percent of erythroid cells were polychromatic while 35 percent were orthochromatic.

In this study, about 48 percent of the total erythroid cells counted in the 11 subjects were megaloblasts. These megaloblasts constituted about 17 percent of the total nucleated cells in the marrow. Megaloblastic changes in the bone marrow of SCA patients was first reported in 1953 when Zueler and Rutzky[5] described a one-year old child with sickle cell anaemia, severe diarrhoea and megaloblastic bone marrow changes. It was reasoned that temporary malabsorption occasioned by the severe diarrhoea could have precipitated negative folate balance. A two-year retrospective study in Nigeria found evidence of megaloblastic changes in 11 percent of 405 newly referred cases with sickle cell anaemia.[20] The findings in that report suggested that megaloblastic changes may not be a universal finding in the bone marrow of SS disease patients. Our present experience tends to suggest the contrary. All the 11 steady state SCA patients studied had megaloblasts in the bone marrow although the proportions varied. The subjects with the least and highest number of megaloblasts had 6.7 percent and 73.7 percent of their erythroblasts to be megaloblasts, respectively. If megaloblasts are found commonly in the bone marrow of steady state SCA patients as presently reported, there will be a need to develop a definition of the level of megaloblasts that constitutes megaloblastic crisis in SCA patients. A reference range (RR) defined by RR = x ± 3SE was calculated and the result was 29 percent - 67 percent. This result may imply that proportion of megaloblasts in the marrow of SS disease patients will be more than 67 percent during megaloblastic crisis. If megaloblast percentage is calculated out of total nucleated cells in the marrow (not as a percentage of erythroblasts), RR will vary between eight percent and 26 percent.

Thus, megaloblastic changes occured in the bone marrow of all the SCA patients in steady state studied even though they were on folic acid supplements. The finding of mean cell volume in the upper range of normal is consistent with the documented megaloblastosis. All the subjects were anaemic (PCV range, 18-23) and had megaloblasts in the marrow. The PCV values did not relate to the degree of megaloblastosis perhaps because the subjects were all anaemic. The cause of this megaloblastosis requires further studies. Such studies should seek, among others, to answer the following questions: (i) could there be deficiencies of other vitamins that need to be corrected even in the steady state of SCA patients? and (ii) could there be a state of chronic malabsorption of vitamins in SCA patients?

The mode of management of patients with megaloblastic arrest of the marrow may not be influenced by this study. However, it is pertinent to note that the presence of megaloblasts in the bone marrow sample of a SCA patient in anaemic crisis, is diagnostic of a megaloblastic arrest only if the megaloblasts are in excess of 26 percent of total nucleated bone marrow cells.

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