To Evaluate the Role of Bone Marrow Aspiration Cytology in Megaloblastic Anaemia

Authors
Dr M.A. Sameer¹, Dr Swapnil Galat², Dr S.A. Deshpande³, Dr Manisha Ahuja⁴
¹Associate Professor, Dept. of Pathology, Dr. SCGMC Nanded
²Resident, Dept. of Pathology, Dr. SCGMC Nanded
³Professor and Head, Dept. of Pathology, Dr. SCGMC Nanded
⁴Resident, Dept. of Pathology, Dr. SCGMC Nanded

Abstract
Background: Bone marrow aspiration plays a major role in the diagnosis of various haematological disorders. Bone marrow aspiration cytology is a proven method for the evaluation of haematological conditions, malignancies, storage disorders and some chronic systemic conditions. Peripheral blood smear examination and other routine laboratory assays are not always sufficient for diagnosis of various diseases which affect the blood and bone marrow. Bone marrow aspiration is useful in making out better individual cell morphology. This study was observational study carried out in the department of pathology of Dr. SCGMC, Nanded over a period of 2 years from May 2015 to May 2017. Bone marrow aspiration along with serum vitamin B12 levels were carried out in 50 suspected cases of megaloblastic anaemia. The aim of this study was to evaluate the role of bone marrow aspiration cytology and its relation to serum vitamin B12 levels in cases of megaloblastic anaemia.
Keywords: Bone marrow aspiration, Serum vitamin B12 levels, megaloblastic anaemia.

Introduction
Bone marrow aspiration cytology is a proven method for the evaluation of haematological conditions, malignancies, storage disorders and some chronic systemic conditions. Peripheral blood smear examination and other routine laboratory assays are not always sufficient for diagnosis of various diseases which affect the blood and bone marrow. Bone marrow aspiration is useful in making out better individual cell morphology. Bone marrow aspiration always gives important information of reaction of haematopoietic tissue in various conditions in addition to findings of blood sample as the bone marrow can be affected by both haematological and non-haematological disorders.
Bone marrow sample can be obtained by aspiration or trephine biopsy, the former being less invasive of the two. There are different sites from which marrow can be sampled. The most preferred site is the posterior superior iliac crest and the selection of this site has the advantage that if no material is aspirated, a trephine biopsy can be performed immediately. Other sites are: anterior iliac crest, manubrium or the first or second part of the body of the sternum. In infants, marrow can be obtained from the medial aspect of
upper end of tibia just below the level of the tibial
tubercle.

Macrocytosis is defined as a mean corpuscular
volume greater than 110 fl, occurs in 3% of
general population. The most common etiologies
are alcoholism, vitamin B12 and folate
deficiencies and medications.

Megaloblastic anaemias are a group of disorders
in which rate of DNA synthesis is retarded while
RNA synthesis is normal causing impaired cell
growth. These usually results from deficiency of
vitamin B12 and/or folic acid resulting in
impaired DNA synthesis. It is characterised by
one or more peripheral cytopenias, oval
macrocytosis, iron overload, erythroid
hyperplasia. When the peripheral smear is
nonmegaloblastic, the reticulocyte count helps
differentiate between drug or alcohol toxicity and
hemolysis or haemorrhage, liver disease and
primary bone marrow dysplasia.

The aims and objectives of the study were:

- To study the bone marrow findings in
cases of megaloblastic anaemia
- To correlate bone marrow findings and
serum vitamin B12 levels in
megaloblastic anaemia.
- To study the incidence of megaloblastic
anaemia according to age and sex.

Methods
This was a hospital based cross sectional
analytical observational study carried out in
department of pathology at Dr. SCGMC, Nanded
over a period of 2 years from May 2015 to May
2017.

Case selection was based on clinical features and
supported by laboratory evidence. Detailed
clinical history was taken and thorough clinical
examination was performed in each case.
Complete blood count by automated cell counters,
peripheral smears were done and bone marrow
aspiration was performed in selected cases. Cases
were further evaluated for serum vitamin B12
levels using chemiluminescent assay.

Inclusion criteria in our study were as follows:

- Patients having clinical features suggestive
of megaloblastic anaemia.
- Patients having Hb < 9 gm % and MCV
>100 fl on automated cell counters.

Exclusion criteria in our study were as follows:

- Patients having associated iron deficiency.
- Patients having dry tap or bloody tap on
bone marrow aspiration.

Blood samples of patients were collected in
EDTA vacutte which was processed through
automated cell counter and haematological
parameters were obtained out of which
haemoglobin, mean corpuscular volume (MCV)
were included in this study. MCV values are
shown in table no. 1. Peripheral smear was
stained by leishman stain for all cases. Bone
marrow aspiration was carried out in selected
cases after obtaining written consent from patient
or guardian.

For bone marrow aspiration a needle with strong,
wide base, short bevelled with stillete and
adjustable guard was used. Posterior iliac crest,
anterior superior iliac spine and sternum are sites
of choice for aspiration.

Procedure
The procedure for bone marrow aspiration was
explained and written informed consent for was
taken from the patient or patient’s relative. The
procedure was performed in a sterile manner.
Local anaesthesia was infiltrated and needle was
introduced in the body cavity and fitting syringe
of 10ml was attached. Strong brief suction was
applied to withdraw 0.3-0.5ml of marrow aspirate.
Aspirated material was immediately placed on
slide and smears were prepared. After air drying
smears were fixed with methanol. The smears
were stained by leishman’s stain and field stain.
Bone marrow examination was done of all smears
and final reports were prepared.

Results
Bone marrow films were examined and reported
in a systemic manner for cellularity, M:E ratio, a
differential count were done along with
morphology of various lineages and finally findings were interpreted with relation to clinical and haematological features and other laboratory findings.

50 patients were included in the study 34 were females and 16 were males. The age range of patients was from 18 years to 55 years. Cases were arranged according to age group in Table:2. Bone marrow aspiration was carried out from posterior aspect of iliac crest in all cases. The bone marrow smears were hypercellular in 37 cases, normocellular in 13 cases.

The most common presenting symptom was generalised weakness, followed by easy fatiguibility and neurological involvement. Clinically pallor was the most common finding followed by hepatosplenomegaly. The frequency of symptoms and signs are listed in table 3 and 4 respectively.

Peripheral blood smear findings: In most cases there is a macrocytic anaemia with oval macrocytes being particularly characteristic. Some degree anisopoikilocytosis is usual. When anaemia is severe there are striking morphological abnormalities like tear drop cells, basophilic stippling and Howell jolly bodies. Hypersegmented neutrophils are usually present. In severe megaloblastic anaemia leucopenia and thrombocytopenia also occur.6

Bone marrow aspiration findings: Incidence of findings of bone marrow examination concluded and incorporated in the study. In 37 cases the most consistent finding was erythroid hyperplasia and thus reversal of M:E ratio from 1:2 to 1:4. The hallmark in the marrow was preponderance of megaloblasts. Megaloblasts are mostly early megaloblasts with open sieve like nuclear chromatin with basophilic cytoplasm. Nuclei of megaloblasts demonstrated features of dyserythropoesis like nuclear budding, irregular nuclei. Myeloid series showed predominance of giant metamyelocyte and giant band or stab forms. Giant metamyelocytes have misshapen nuclei, abnormal chromatin and staining character. When megaloblastic features in erythroblasts are partly or largely masked by co-existing iron deficiency; then detection of giant metamyelocytes are diagnostically important because they are constantly present in the smears.6 Megakaryocytes were normal in number and had normal morphology.

All these cases were further evaluated for serum vitamin B12 levels using chemiluminescent assay. Serum vitamin B12 levels were found to be decreased in 37 patients. Thus findings of megaloblastic anaemia on bone marrow aspiration were correlated with serum vitamin B12 levels.

**Table 1: MCV levels**

<table>
<thead>
<tr>
<th>MCV in fl</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>101-105</td>
<td>5</td>
</tr>
<tr>
<td>106-110</td>
<td>8</td>
</tr>
<tr>
<td>&gt;110</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 2: Age group distribution of study**

<table>
<thead>
<tr>
<th>Age in years</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-25</td>
<td>15</td>
</tr>
<tr>
<td>26-40</td>
<td>30</td>
</tr>
<tr>
<td>41-55</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 3: Frequency of symptoms**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised weakness</td>
<td>38</td>
</tr>
<tr>
<td>Fatigue</td>
<td>35</td>
</tr>
<tr>
<td>Neurological</td>
<td>15</td>
</tr>
<tr>
<td>Jaundice</td>
<td>5</td>
</tr>
<tr>
<td>Recurrent fever</td>
<td>5</td>
</tr>
<tr>
<td>Weight loss</td>
<td>9</td>
</tr>
</tbody>
</table>

**Table 4: Frequency of signs**

<table>
<thead>
<tr>
<th>Signs</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallor</td>
<td>48</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>30</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>20</td>
</tr>
<tr>
<td>Skin hyperpigmentation</td>
<td>14</td>
</tr>
<tr>
<td>Neurological signs</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 1 Peripheral Smear showing Macroovalocytes
Fig. 2 Peripheral smear showing hypersegmented neutrophil

Fig. 3: Bone marrow aspirate smear showing erythroid hyperplasia

Fig. 4: Bone marrow aspirate smear showing megaloblasts having sieve like nucleus

Fig. 5: Bone marrow aspirate smear showing giant stab form

Discussion
Anaemia is the most common disease diagnosed by bone marrow cytology study. Megaloblastic anaemia usually occurs due to deficiency of vitamin B12 or folic acid. Some patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) also have megaloblastic erythropoiesis. Along with peripheral blood smear and bone marrow aspiration findings, further tests that should be done in patients with megaloblastic anaemia are assays of serum vitamin B12 followed by tests for auto antibodies and a schilling test.

The term macrocytosis refers to a blood condition in which red blood cells (RBC) are larger than normal. Macrocytosis is reported in terms of mean corpuscular volume (MCV). Normal MCV values range from 80-100 femtoliters (fl) and vary by age and reference laboratory.

Macrocytosis can be identified by reviewing peripheral blood smears and/or by automated RBC indices. The peripheral blood smear is more sensitive than RBC indices for identifying early macrocytic changes because the MCV represents the mean of the distribution curve and is insensitive to the presence of small numbers of macrocytes. In case of cobalamin deficiency, with its slow progression, macrocytosis precedes anaemia by months.

The common feature of all megaloblastic anaemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow and other tissues. The main clinical feature in more severe cases is that of anaemia. Anorexia is usually marked, and there may be weight loss, diarrhoea or constipation. Other features include glossitis, angular cheilosis, mild fever, unconjugated jaundice and reversible skin hyperpigmentation.

In our study maximum patients were females. Most of the patients were found in the age group of 26-40 years (60%). In our study MCV was slightly raised in 5 patients with levels between 101-105 fl, in 8 patients levels were between 106-110 fl and 37 cases have levels >110 fl.
In patients with elevated MCV values, peripheral blood smear examination was carried out. Macrocytosis and Macroovalocytosis along with hyper segmented neutrophils were the most common findings on peripheral smear. All cases with elevated MCV values underwent bone marrow aspiration. On bone marrow aspiration, characteristic features of megaloblastic anaemia like hypercellular marrow with erythroid hyperplasia with preponderance of megaloblasts along with giant metamyelocyte were commonest findings in 37 cases. In 13 cases the bone marrow smears were normocellular showing normal features.

After bone marrow aspiration, evaluation for vitamin B12 levels was done which were found to be decreased in 37 patients. Thus bone marrow aspiration findings were correlated with serum vitamin B12 levels. Thus, the diagnosis of megaloblastic anaemia was confirmed in 37 patients both on bone marrow examination and serum vitamin B12 levels. In other 13 patients in which MCV levels were slightly raised, bone marrow showed normal features and serum vitamin B12 levels were not decreased the other causes of macrocytosis like alcoholism, medications or liver diseases can be taken into consideration.

**Conclusion**

Bone marrow aspiration cytology is a mildly invasive technique but which can be carried out in patients rapidly and it helps in the confirmed diagnosis of megaloblastic anaemia within short period.

**References**