ORIGINAL ARTICLE

Bone Marrow Aspirrration Vs Bone Marrow Trephine Biopsy in Dignosis of Aplastic Anemia

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Abstract

The current study was conducted to evaluate the efficacy of bone marrow trephine biopsy and Bone marrow aspiration in diagnosis of aplastic anemia. This cross sectional study was done in Department of Pathology for a period of 10 years. A total of 78 cases were enrolled in the study. After proper history and physical examination, patients were subjected to detailed haematological examination. Severity of aplastic anemia was defined on the basis of peripheral blood picture, trephine biopsy and corrected reticulocyte count. A total of 78 cases were included, 61.5% were male and 38.5% were female patients. There was bimodal age of presentation but the most common affected age group was in 11-20 years. Peripheral blood smears showed normocytic, normochromic blood picture in 67.9%. Macrocytic predominance was seen in 21.8%. 50.6 % had severe aplastic anemia and 49.3% were having non severe aplastic anemia. Majority of bone marrow aspirates 85.3% correlated with bone marrow trephine biopsy. Aplastic anemia is seen in all age groups. Bone marrow trephine biopsy is superior to bone marrow aspirate in patients especially where marrow particles show variable cellularity.

Key Words

Aplastic Anemia, Bone Marrow Aspiration, Trephine Biopsy

Introduction

Aplastic anaemia (AA) is defined as bone marrow failure syndrome characterized by peripheral blood pancytopenia and bone marrow hypoplasia.(1) It is a rare disease which results in morbidity and mortality at a young age (2). The incidence ranges from 2-6 new cases per 1 million.(2, 3) It is about 2-3 fold higher in Asia. It is important to distinguish aplastic anemia from other causes of pancytopenia such as 'aleukemic leukemia' and myelodysplastic syndrome.(1-9) Recent evidence suggests an immune-mediated process underlying pathogenesis of aplastic anaemia4. Bone marrow aspirate alone is not sufficient to confirm the diagnosis, as hypocellular marrow devoid of hemopoietic cells often yields a little material (known as, dry tap or blood tap). A trephine biopsy is, therefore, indispensible in diagnosis of aplastic anaemia. Marrow biopsies are required to document that a hypocellular marrow aspirate represents hypoplasia or aplasia and to exclude other conditions that may infiltrate, replace or suppress normal marrow cells. This study was conducted to evaluate the efficacy of bone marrow aspirate and bone marrow trephine biopsy in patients of aplastic anemia in a cohort of North Indian patients.

Material and Methods

This cross sectional study was carried out in Department of Pathology for a period of ten years at Government Medical College Jammu, to evaluate efficacy of bone marrow aspirate and bone marrow trephine biopsy in patients of aplastic anemia.

A total of 78 cases were studied. Out of these, 72 cases were retrospective, while 6 cases were prospective. Source of information for retrospective cases included patient's case files from Medical Record Department. For prospective cases, all such patients with history of clinical features like pallor, fatigue, bleeding in the form of bruising or petechiae, persistent fever, oral and throat ulcers were taken up for detailed hematological analysis.

The inclusion criteria:

1. Hb <13.5gm (males); Hb <11.5gm (females)
2. Total leukocyte count <4000/mm3
3. Platelet count <1.5 lakh/mm
4. Reduced reticulocyte count

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5. Peripheral blood film (PBF) showing no abnormal or immature cells of either granulocytic or erythroid series and absence of hypersegmented polymorphs or macropolycytes.

6. Buffy coat smear showing absence of abnormal or immature cells.

All such patients were taken up for detailed hematological analysis including bone marrow aspiration and bone marrow trephine biopsy. History of jaundice as well as any viral illness was also recorded. A proper consent was taken from the patients and/or their attendant. The blood sample from the patients were tested for Complete Blood Counts (CBC); including Haemoglobin (Hb), Total leukocyte count (TLC), Platelet count and detailed peripheral smear. PBF was stained with Romanowsky stains (Leishman stain) and peripheral smear examination was carried out for differential leukocyte count (DLC), identification of any blasts, presence of dysplasia. Percentage reticulocyte count was done by staining the blood film with supravital stain 5 and reticulocyte index was also calculated.

All the patients were subjected to bone marrow examination. Bone marrow aspiration was performed on posterior superior iliac spine under all the aseptic precautions by using Salah's aspiration needle. Skin, subcutaneous tissue and periosteum overlying the selected site was infiltrated by 2% xylocaine (2-5 ml) after sensitivity testing. With boring movement, needle was passed perpendicularly into the cavity of the ilium at the centre of the oval posterior superior iliac spine. Stilette was removed when the bone was penetrated. 10 ml syringe was attached and 0.2 ml marrow contents were sucked into the syringe. 3-5 cm long marrow smears were prepared by using 2 cm wide smooth edged glass spreader. The marrow fragments were dragged behind the spreader and trail of cells was left behind. After drying, the smears were stained with May-Grunwald Giemsa (MGG) stain and Perl's stain. Bone marrow aspiration smears were looked for cellularity, M: E ratio and morphology of all cell lineages and iron stores.

Marrow Trephine Biopsy was done in all the cases presenting with isolated or bicytopenia with either hypocellular marrow particles on bone marrow aspirations, dry tap, blood tap or smear showing inadequate material on bone marrow aspiration. It was performed by using the Jamshidi needle from posterior iliac spine taking all aseptic precautions. Trephine specimen was obtained by inserting the biopsy needle into the bone and using to and fro rotation to obtain a core of tissue. For Imprint smears, the bony core was gently dabbed or rolled across the slide, which was then fixed and stained with MGG stain. Specimen obtained was fixed in Bouin's fluid for 12 to 48 hours prior to dehydrating and embedding in paraffin. Bony tissue was decalcified using 5% nitric acid and keeping for 1-4 days. Formalin fixed tissue was dehydrated with ascending grades of alcohol, cleared in xylene and finally embedded in paraffin. 3-5 µ thick paraffin sections were cut on a rotary microtome, dewaxed and stained with Haematoxylin and Eosin stain 6 and reticulin staining was done using a silver impregnantion technique . Trephine biopsy specimen <1.5 cm was considered as inadequate, while >1.5 cm was labelled as adequate biopsy specimen. Comprehensive details including marrow architecture, overall cellularity, and pattern of involvement, foci of cellular areas (hot spots of clustered erythroid precursors), reticulin fiber density and thickness were studied. Cellularity assessment was based on visual examination and graded into two groups for all identified hypocellular samples. i) Less than 25% cellularity ii) 25-50% cellularity.

The cases of aplastic anemia thus diagnosed were sub classified as non severe, severe and very severe aplastic anemia. The modified Camitta criteria are used to assess severity.7 Severe AA; Marrow cellularity < 30% residual haematopoietic cells), plus at least 2 of: a) neutrophils < 0.5 x 109 /l; b) platelets < 20 x 109 /l; c) reticulocyte count < 20 x 109 /l reticulocyte count) o Very Severe AA; As for Severe AA but neutrophils < 0.2 x 109 /L o Non severe AA; AA not fulfilling the criteria for severe or very severe AA.

Results

A total of 6346 bone marrow aspirations were done in our department during the study period. Among them in 97 cases, bone marrow examination showed hypoplastic marrow. 78 patients with pancytopenia and hypoplastic marrow who had no hepatosplenomegaly were included in our study. All 78 cases were investigated with both bone marrow aspiration and biopsy. Aplastic anemia was diagnosed in 75 cases. The age group of patients affected ranged from 6 to 75 years. The most common affected age group was in the range of 11 to 20 years. Male accounted for 61.5% cases and female 38.5% cases with a male: female ratio of 1.6:1. Majority of patients were severely anaemic with 52.6% of patients having hemoglobin concentration <6 gm/dL. Total erythrocyte count was in the range of 1-2 million/mm3 in 78% of patients, while 21.8% patients had count between 2-3 million. The total leukocyte count was <2000/mm3 in majority (46.2%) of the patients, while 29.3% patients

Table 1. Frequency of histopathological alteration in gallbladder mucosa

Aplastic anemia	Marked hypoplasia (<25% of normal cellularity)	Moderate hypoplasia (25-50% of normal cellularity)
Severe aplastic anemia (n=38)	20	18
Non-severe aplastic anemia (n=37)	30	7

Table 2. Bone marrow aspirate: correlation with bone marrow biopsy (n = 75)

Concordance with bone marrow biopsy No. (%)	Discordance No. (%)	Inadequate for opinion No. (%)
64 (85.3)	4 (5.3)	7 (9.4)

[Out of 78, biopsy of 3 cases were inadequate (<1.5 cm) for assessing cellularity.]

Table 1b Hematological parameters and severity of aplastic anemia

Hematological parameters	Bone marrow findings
Absolute neutrophil count <500/mm^3 (n= 37)	34 (SAA) 3 (NSAA)
Platelet count <20,000/mm^3 (n=37)	36 (SAA) 1 (NSAA)
Corrected reticulocyte count <1% (n=63)	31 (SAA) 32 (NSAA)

normocytic, normochromic blood picture in 67.9%. Macrocytic predominance was seen in 21.8%. On bone marrow aspiration, majority of the cases (85.8%) had hypocellular marrow with increase fat spaces and the cellularity comprised of mostly lymphocytes, reticulum cells, plasma cells and mast cells. Four cases (5.3%) had marrow with variable cellularity. In 7 cases (8.9%), bone marrow was diluted with sinusoidal blood, hence termed

Table 3. Correlation of bone marrow aspirate with bone marrow biopsy cellularity

Bone marrow aspirate	Bone marrow trephine biopsy: Hypoplasia No.	Bone marrow trephine biopsy: Inadequate sample for cellularity No.	McNemar Test (Inference)
Hypocellular marrow	64	3	Value = 4.6; p=0.02; Significant
Variable cellularity, some particles hypocellular and others showing normal cellularity	4	0	
Inadequate for opinion	7	0	

Sensitivity = 89.33 (range 79.53 - 94.95) *Specificity = 100% (range 30.99 - 100%)*
Positive predictive value =100% (range 93.24 - 100%) *Negative predictive value = 27.27 (range 7.32 - 60.68)*
Positive likelihood ratio = Not applicable *Negative likelihood ratio = 0.106 (range 0.055 - 20.53)*

Table 4. Correlation of bone marrow findings and complete blood count parameters

No. of cases	Hb range (gm/dL)	TEC range (million/mm^3)	TLC range (per mm^3)	Platelet count range (per mm^3)	Trephine biopsy findings (hypoplasia)
58	3.2-8.2	1.02-2.82	1000-3900	10,000-80,000	Marked = 35; Moderate = 30; Inadequate = 3
4	3.5-7.2	1.05-2.14	1500-3200	5000-72,000	Marked = 4
7	3-9	1.2-2.8	1600-3800	5000-28,000	Marked = 4; Moderate = 3
6	4.2-7.5	1.24-1.56	1400-3700	5000-90,000	Marked = 4; Moderate = 2
1	5.2	1.45	1600	15,000	Marked
1	6.2	1.48	2800	50,000	Marked
1	4.5	1.2	1200	10,000	Marked

had counts between more than 3000/mm3, but less than 4000/mm3, 24.3% had counts between 2000-3000/mm3. Absolute neutrophil count <500/mm3 was seen in 49.3%, while corrected reticulocyte count <1% was seen in 84.0%.The platelet count in majority (47.6%) of the patients was <20,000/mm3, while 43.5% patients had count between 20,000-50,000/mm3. PBF showed inadequate for opinion. Iron stores were adequate in majority of cases (n=72), while increased iron stores was observed in remaining 6 cases. In bone marrow biopsies, hypoplastic marrow was seen in 75 cases. In 3 cases sample was inadequate (bone marrow biopsy length <1.5 cm) for assessing cellularity. 50 patients (60.6%) had marked hypoplasia, while marrow was moderately

hypoplastic in 25 (33.4%) patients, Residual hematopoiesis in erythroid series showed depression in majority (94.6%) patients. Hot spots of clustered erythroid precursors with some of them showing megaloblastic change were seen in 5.4% patients. Myeloid series were absent in 64% of patients, while residual hematopoiesis showed depression in 33.3% of patients. 2.7% patients showed foci of remnants of myeloid precursors in erythroid hot spots. Megakaryocytes were absent in bone marrow biopsy specimens in 66.6% patients. Depression of megakaryocytes was seen in 33.4% patients. Stromal features like hemorrhage were noted in 9.3% patients. Cells comprising of lymphocytes, plasma cells and mast cells was seen in 90.7% patients. No clustering of lymphocytes, lymphocytic nodule was seen in any of the 75 patients. 75 bone marrow biopsy specimens were stained with reticulin stain. Reticulin grades were within normal limits in all the patients.

38 out of 75 patients (50.6%) had severe aplastic anemia. Rest (n=37) were graded under non-severe aplastic anemia. (*Table 1a & b*). We compared the concordance and discordance of bone marrow aspirate with bone marrow biopsy in 75 cases. Majority of bone marrow aspirates i.e. 64 (85.3%) correlated with bone marrow trephine biopsy findings. In 7 (9.4%) cases, aspirate was inadequate for opinion because of dilution of marrow with sinusoidal blood. Bone marrow aspirate in 4 (5.3%) did not correlate with bone marrow trephine biopsy findings. Bone marrow aspirate showed a statistically significant correlation with bone marrow trephine biopsy. (*Table 2 & 3*). Complete blood count parameters like hemoglobin, total erythrocyte count, total leukocyte count and platelet count were fairly predictable of the degree of marrow hypoplasia in majority of the cases. (*Table 4*)

Discussion

Aplastic anaemia is a rare disease, thus its true incidence is uncertain. In the present study, 6346 bone marrow aspirations were done and 97 cases were reported as hypoplastic marrow. Out of 97, 75 cases were reported as aplastic marrow on trephine biopsy. In the present study, maximum number of patients (43.6%) was seen in the age group of 11-30 years, followed by 17.9% seen in age above 51 years. The age of onset presented a definite decline with progressive age. Davies and Walker and Panahi *et al.* in their study concluded that the incidence of aplastic anemia varies with age bimodally (8, 9). Male to female ratio in the present study was 1.6:1 Male: female ratio was comparable with the others studies of Shah *et al.* and Ehsan *et al.*(10, 11) In 67.9% cases, blood smear showed red blood cells of normocytic and normochromic . . . . . *et al.* reported 88.9% with normocytic and normochromic morphology10. The corrected reticulocyte count ranged from 0.2 to 1% (mean 0.5%). Shah *et al.* Reported corrected reticulocyte count ranged from 0.2 to 0.9% with a mean of 0.5% (10). In present study, majority of the patients (78.2%) had total erythrocyte count between 1 to 2 million, while in a study by Haldar *et al.* ,majority of the patients (78.95%) had total erythrocyte count between 3 to 4 million/mm3.(12)

In the present study, out of 66.6% of bone marrow biopsies showed marked hypoplasia and 33.4% showed moderate hypoplasia. In the present study, marked hypoplasia was seen in 60.6% while 33.4% had moderate hypoplasia. Ehsan et al. Reported marked hypoplasia in 38% cases (11) All the three hemopoietic lineages are affected to a variable extent. In the present study, majority of the erythroid precursors, myeloid precursors and megarkaryocytic precursors were depressed. Ehsan et al. found erythropoiesis, myelopoiesis and megarkaryopoiesis markedly depressed in 92% cases (11). However in 4 patients, erythroid precursors were present in the hot pockets (cellular foci). Kansu and Erslev in their study found foci of intense hematopoietic activity (hot pockets) in patients with chronic severe aplastic anemia.(13) The presence of persistent 'hot pockets' present a conceptual challenge since these pockets contain multipotential stem cells capable of differentiation and self renewal, but incapable of repopulation of the bone marrow.

Megarkaryocytes were absent in majority of cases (66.6%) while Myeloid series were absent 64%. Stromal features like hemorrhage, disruption of sinusoidal wall with oedema and necrosis of capillaries are reported by Velde and Haak in a study (14). In the present study, hemorrhage was seen in 9.3% cases. Bone marrows of patients, including those of remission, frequently show inflammatory cells (lymphocyte usually intermingled with other inflammatory cells such as plasma cells, mast cells and macrophages in loosely structured infiltrates in hematopoietic areas) (14). Frisch and Lewis concluded in their study that high proportion of lymphocytes in the marrow denotes a serious prognosis.(15) In the present study, bone marrow showed cells comprising of lymphocytes, plasma cells and macrophages in 90.7%

cases. Reticulin staining is one of the important features in distinguishing a hypoplastic marrow in cases of aplastic anemia from hypoplastic myelodysplastic syndrome and acute myeloid leukemia, both of which frequently show increase in reticulin fibres All the cases in the present study showed a reticulin grade up to 1.

Milosevic *et al.* analysed marrow biopsy specimens of 33 patients in their study and concluded that histopathologic examination of bone marrow biopsy is necessary for definitive diagnosis of aplastic anemia.(16) Cellularity estimation in bone marrow based on biopsy and histopathologic examination is more reliable than that made by bone marrow aspiration. The peripheral blood film and complete blood count findings correlated fairly well with that of bone marrow findings.

In our study, the correlation between bone marrow aspirate and bone marrow trephine biopsy was statistically significant (p=0.02). To our knowledge, no study in the literature has so far been conducted to assess the correlation between bone marrow aspirate and trephine biopsy in aplastic anemia. Using the above criteria, 50.6% out of 75 patients fulfilled the criteria for severe aplastic anemia. Those not fulfilling 49.4% were graded as non-severe aplastic anemia. Our study showed prevalence of severe aplastic anemia more than that reported by Goswami *et al.* (17). Majority of cases of severe aplastic anemia (47.4%) were seen in age group 11-20 years in the present study. Ehsan et al. reported 69% of severe aplastic anemia in age group below 25 years11

Conclusion

Bone marrow trephine biopsy is superior to bone marrow aspirate, especially in a case where marrow particles show variable cellularity. Peripheral blood showing pancytopenia, reduced red cell density, normocytic normochromia or slight macrocytosis (round macrocytes) with absence of both erythroid and myeloid precursors in a patient who is anemic without organomegaly or lymphadenopathy is sufficient to make a provisional diagnosis of aplastic anemia at the time of admission. Any patient of pancytopenia with above mentioned features should be recommended for bone marrow studies to confirm the diagnosis of aplastic anemia and thus avoids unnecessary delay in such patients.

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