High frequency of infection with tuberculosis as the most common cause of bone marrow necrosis: a study from tertiary care centre in northern India and their clinico-pathologic analysis

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Abstract Bone marrow necrosis (BMN) is seen in various malignant and non-malignant disorders. The relative frequency ranges between 0.37 and 6.5 % in western countries. The aim of this study is to analyze various etiology and prevalence at our tertiary care center and to compare our clinico-pathologic profile of this uncommon entity to western data. This is a retrospective observational study done in the Department of Hematology, tertiary care centre in northern India over a period of 21 months. Various clinical features and laboratory parameters of the cases showing BMN were noted from hospital records. Bone marrow trephine biopsies with BMN were graded semi-quantitatively according to the criteria laid down by Maisel et al. grading system into three grades I, II, and III as area of the biopsy showing necrosis like <20, 20–50, and >50 %, respectively. Bone marrow necrosis was observed in 1.8 % of bone marrow biopsies studied for various clinical indications over the study period. Median age was 35 years with male to female ratio of 3.4:1. Underlying non-malignant disease was seen in 64.4 % cases with tuberculosis as the most common etiology (59.32 %). Among malignancies, hematolymphoid malignancies were observed in 23.72 % cases. On semi-quantitative grading, bone marrow biopsies revealed grade I, II, and III necrosis in 62.71, 18.64, and 18.64 % of cases, respectively. BMN is rare clinico-pathological entity with a poor prognosis. Although the etiology of BMN is varied, this differential possibility should be kept in mind in any patient presenting with bicytopenia or pancytopenia especially in the setting of infection and malignancy. Tuberculosis should

always be an important differential diagnosis in developing countries like India, before considering malignancy.

Keywords Bone marrow necrosis · Tuberculosis · Hematologic malignancies

Introduction

Bone marrow necrosis (BMN) is a relatively uncommon histomorphological feature noted during the bone marrow examination and is a unique clinico-pathologic entity [1]. This entity was described for the first time by Wade and Stevenson, in 1941, in a patient with sickle cell disease who died of cerebral infarction [2]. Diagnosis is done on characteristic cytomorphological pattern of the bone marrow aspiration (BMA) and/or bone marrow biopsy (BMB). Cytology reveals pyknotic cells which are barely recognizable in an amorphous eosinophilic proteinaceous background while histopathological examination of BMB shows disrupted marrow architecture with loss of fat spaces [3]. Although autopsy reports show a higher prevalence of this disorder, the prevalence of BMN is highly variable and is a relatively rare entity diagnosed during life [4]. The etiology of BMN is diverse with most of the western literature mentioning hematological malignancy as the commonest cause [5–9]. The non-neoplastic causes of BMN include sickle cell disease, infections, sepsis, and chemotherapeutic drugs used in management of hematological malignancies. Rare causes such as anorexia nervosa, hemolytic uremic syndrome, anti-phospholipid antibody (APLA) syndrome, DIC associated with disseminated metastasis, and hyperparathyroidism are also associated with BMN [10]. The aim of the present study was to analyze the prevalence of BMN among the BMA/BMB routinely examined at a tertiary care center in a developing country for various hematological

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and non-hematological indications. The study also aims at identifying the diverse etiology and the range of histomorphological features associated with BMN and to compare the same with that of western literature.

Materials and methods

This was a retrospective observational study done in the Department of Hematology, tertiary care centre in northern India. It included review of all BMB performed between January 2012 and September 2013. BMB showing BMN during life (ante-mortem cases) were included in this study. However, BMB showing only granuloma without caseous necrosis and BMN following chemotherapy/ radiotherapy were excluded from analysis. In all cases, peripheral smear (PS), BMA, BMB findings as well as immunophenotyping (by flowcytometry and immunohistochemistry) data wherever applicable were obtained from laboratory records, and detailed clinical findings were taken from outpatient department.

Bone marrow aspiration and trephine biopsy were done from posterior superior iliac crest after obtaining informed written consent from the patients. Air-dried smears were used for PS and BMA, stained with Jenner-Giemsa stain. Bone marrow biopsies were ranging between 0.5 and 4.3 cm size with the median size of 1.8 cm. In some cases, two different BMB were performed simultaneously from different sites, if focal nature of the disease was considered. In all cases, BMB were received in 10 % formalin with overnight fixation, processed, and decalcified in formic acid, and paraffin-embedded blocks were used to take 3- μ m section and stained with hematoxylin and eosin (H & E) stain. Special stains like acid-fast stain (Ziehl-Neelsen stain), periodic acid Schiff (PAS) stain, and Grocott's silver stain were performed in all suspected infectious diseases. In acute leukemia and lymphoma cases, immunophenotyping was done by flow cytometry; immunohistochemistry (IHC) was done for identification and sub-classification of the disease in selected cases. For IHC, 3- μ m section was taken on poly-L-lysine coated slides.

Subsequently, all the cases were classified into three broad categories like hematologic malignancies, solid organ malignancies, and non-malignant disease. Bone marrow trephine biopsies with BMN were graded according to the criteria laid down by Maisel et al. [1], semi-quantitatively into three grades I, II, and III showing <20, 20–50, and >50 % area of the biopsy showing necrosis, respectively. The clinico-pathologic variables along with BMN were studied in these three categories. Statistical analyses were performed by Fischer's exact test or chi-square test using SPSS software, and *p* value less than 0.05 was considered as statistically significant.

Results

Prevalence

A total of 3500 bone marrow biopsies were received in the abovementioned period. In majority of the cases, BMB was performed for the suspected malignancy (2413 cases, 68.94 %). In the remaining 1087 cases (31.05 %), BMB was performed for suspected infections with tuberculosis (TB) as one of the cause. Among these, BMN was seen in 63 cases accounting for 1.8 % prevalence in our tertiary care centre in northern India. Median age was 35 years (range 13–76 years), and male to female ratio was 3.4:1. Among these 63 cases of BMN, four cases were excluded because these cases received either chemotherapy or radiotherapy. Hence, only 59 cases were further evaluated.

Clinical findings

The most common presenting symptom was fever seen in 77.96 % of all cases followed by weakness and easy fatigability (66.10 %). Pallor (88.14 %) was the most common clinical sign followed by significant lymphadenopathy (27.12 %). Further split up of various clinical findings in three categories and their *p* value were shown in Table 1.

The most common peripheral blood abnormality was anemia seen in 86.44 % among all cases followed by bicytopenia (66.10 %). Hyperleucocytosis was seen only in 4.8 %. Other peripheral blood abnormalities and their *p* value are summarized in Table 2.

Underlying disease association

The various malignant and non-malignant diseases responsible for BMN (Figs. 1 and 2) as well as their grading according to Maisel et al. grading system are shown in Table 3. Underlying non-malignant disease was seen in 64.4 % cases. As expected, in India, TB (55.93 %) was the most common cause of BMN. Tuberculosis co-infection with human immunodeficiency viral (HIV) infection was seen in 3.38 %.

TB cases were diagnosed based on classic morphological features like caseous necrosis with granuloma, supported by acid-fast stain, along with clinico-radiological correlation. Among 35 cases of TB, extra-medullary manifestations were observed as pulmonary involvement in 13 cases, nodal involvement (13 cases), splenic abscess (1 case), renal involvement (2 cases), thoracic vertebral fracture (1 case), and disseminated disease (11 cases). Among cases evaluated for pyrexia of unknown origin, 10 cases had primary TB diagnosis from BMB and subsequently found to have disseminated disease. Two cases of HIV had co-infection with TB. In BMB, 30 cases of TB cases had simultaneous BMN and granuloma and five cases had only BMN. Special stain yielded acid-fast stain

Table 1 Various clinical findings in patients with bone marrow necrosis (BMN) ($n=59$ cases)

Clinical findings	Hematologic malignancy—14 cases (23.72 %)	Solid organ malignancy—7 cases (11.86 %)	Non-malignant diagnosis—38 cases (64.40 %)	<i>p</i> value
Symptoms				
Fever	13 (92.85 %)	1 (14.28 %)	32 (84.21 %)	0.000
Bleeding manifestation	7 (50 %)	2 (28.57 %)	4 (10.52 %)	0.008
Weakness and easy fatigability	10 (71.42 %)	7 (100 %)	22 (57.89 %)	0.094
Bony pain	3 (21.42 %)	3 (42.85 %)	4 (10.52 %)	0.061
Abdominal mass/adenopathy	6 (42.85 %)	4 (57.14 %)	21 (55.26 %)	0.734
Signs				
Pallor	11 (78.57 %)	6 (85.71 %)	35 (92.10 %)	0.328
Icterus	1 (7.14 %)	1 (14.28 %)	4 (10.52 %)	1.000
Significant lymphadenopathy	1 (7.14 %)	2 (28.57 %)	13 (34.21 %)	0.153
Hepatomegaly	5 (35.71 %)	0 (0 %)	7 (18.42 %)	0.174
Splenomegaly	5 (35.71 %)	1 (14.28 %)	9 (23.68 %)	0.606
Bony tenderness	3 (21.42 %)	4 (57.14 %)	4 (10.52 %)	0.014
Petechiae/purpura/ecchymosis	7 (50 %)	2 (28.57 %)	4 (10.52 %)	0.008

p value less than 0.05 was considered statistically significant

positivity in 10 cases only. In the remaining cases, diagnosis was confirmed in correlation with clinico-radiological findings and microbiological investigations. Three cases died within short span of admission because of disseminated disease, particularly one case with TB/HIV co-infection and two cases with BMN grade III without granuloma. Remaining cases were started on anti-tubercular drugs and responded well.

The majority of TB cases with BMN were categorized under grade I BMN (30/38 TB cases) (81.07 %). Three cases of TB (27.27 %) had grade II necrosis, and two cases (18.18 %) had grade III necrosis. Bone marrow biopsies showing only granuloma without any necrotic focus were excluded from the study; however, cases showing marrow necrosis in addition to granuloma were included. The other uncommon causes for non-malignant grade I BMN include one

case each of histoplasmosis and systemic lupus erythematosus (SLE) on steroid treatment, respectively (Table 3).

The most common underlying malignancy was hemato-lymphoid malignancy in 23.72 % cases. At diagnosis, BMN were noted in six cases of acute leukemia, and a case of relapsed B-acute lymphoblastic leukemia (B-ALL) also showed BMN. All leukemia cases were associated with grade III BMN except a case of T-acute lymphoblastic leukemia (T-ALL). Table 3 highlights other hemato-lymphoid malignancies and their BMN grading. Among the solid organ malignancies, metastatic adenocarcinoma of unknown origin in three cases, metastatic squamous cell carcinoma of upper aero-digestive tract origin in two cases, and metastatic neuro-endocrine adrenal malignancy in one case were seen in this study (Table 3). However, even after extensive work up of one case with grade III BMN, etiology could not be identified.

Table 2 Peripheral blood finding in BMN patients ($n=59$ cases)

Peripheral blood findings	Hematologic malignancy—14 cases (23.72 %)	Solid organ malignancy—7 cases (11.86 %)	Non-malignant diagnosis—38 cases (64.40 %)	<i>p</i> value
Anemia ($Hb < 110$ g/L)	11 (78.57 %)	5 (71.42 %)	35 (92.10 %)	0.170
Leucopenia ($TLC < 4 \times 10^9/L$)	7 (50 %)	0 (0 %)	20 (52.63 %)	0.032
Leucocytosis ($TLC > 11 \times 10^9/L$)	3 (21.42 %)	2 (28.57 %)	2 (5.26 %)	0.096
Thrombocytopenia ($Plts < 150 \times 10^9/L$)	12 (85.71 %)	4 (57.14 %)	21 (55.26 %)	0.125
Bicytopenia	11 (78.57 %)	3 (42.85 %)	25 (65.78 %)	0.264
Pancytopenia	4 (28.57 %)	0 (0 %)	11 (28.94 %)	0.223
Leucoerythroblastic blood picture	5 (35.71 %)	6 (85.71 %)	2 (5.26 %)	0.000
Abnormal cells (leukemia/lymphoma spill)	5 (35.71 %)	0 (0 %)	0 (0 %)	0.000

p value less than 0.05 was considered statistically significant

Fig. 1 **a** Bone marrow biopsy (BMB) showing epithelioid cell granuloma with central caseous necrosis in tuberculosis case (H & E stain, $\times 100$). **b** Same case shows necrosis (grade I) and multinucleated giant cells (H & E stain, $\times 400$). **c** Macrophages with engulfed histoplasma in histoplasmosis case (H & E stain, $\times 1000$). **d** Same case shows Grocott's silver stain positivity ($\times 1000$) and mucicarmine stain negative (not shown)

Utility of ancillary tests

Immunohistochemistry (IHC) helped us in reaching diagnosis of one metastatic neuroendocrine malignancy (both synaptophysin and chromogranin positive), three cases of CD20+ DLBCL, and a case of Hodgkin's lymphoma (CD15+ and CD30+) infiltration in BMB. Flow cytometry immunophenotyping data were available for leukemia diagnosis in three cases of B-ALL at diagnosis, two cases of acute myeloid leukemia (AML) at diagnosis, one case each of relapsed B-ALL, T-ALL at diagnosis, and CML-BC, respectively.

Fig. 2 **a** BMB showing cellular and necrotic areas in stage IV Hodgkin's lymphoma case (grade I) (H & E stain, $\times 100$). **b** Multilobated Reed-Sternberg (RS) cell with prominent eosinophilic inclusion like nucleoli (H & E stain, $\times 1000$) in a reactive background. **c** Same case showing CD30 positivity ($\times 400$, IHC)

Discussion

Bone marrow necrosis (BMN) is a rare ante-mortem diagnosis [4, 10–14]. The relative frequency ranges between 0.37 and 6.5 % in western countries [14], 0.4 % in an Indian study [12], and comparable 1.8 % prevalence in our study.

The most common presenting feature among all the cases in our study was fever (75.8 %) followed by weakness and easy fatigability (50 %). Fever ($p=0.000$), bleeding manifestations ($p=0.008$), and bony tenderness ($p=0.014$) were the most significant symptoms and signs among the three categories (Table 1). This is in quite contrast to western study [4], in

Table 3 Etiological diagnosis in patients with BMN and their Maisel et al. grading

Final diagnosis	Maisel et grade I (37 cases) (62.71 %)	Maisel et grade II (11 cases) (18.64 %)	Maisel et grade III (11 cases) (18.64 %)
Hematologic malignancy—14 cases (23.72 %)	T-ALL at diagnosis (1) (2.70 %) RAEB-1 on BMA with BMN on BMB (1) (2.70 %) Hodgkin's lymphoma infiltration (1) (2.70 %) Hepatosplenic T-cell lymphoma infiltration (1) (2.70 %)	Diffuse large B-cell lymphoma infiltration (2) (18.18 %)	B-ALL at diagnosis (3) (27.27 %) AML at diagnosis (2) (18.18 %) Relapsed B-ALL (1) (9.09 %) Diffuse large B-cell lymphoma infiltration (1) (9.09 %) CML-myeloid blast crisis (1) (9.09 %)
Solid organ malignancy—7 cases (11.86 %)	Metastatic squamous cell carcinoma (1) (2.70 %)	Metastatic adenocarcinoma (4) (36.36 %) Metastatic neuroendocrine adrenal malignancy (1) (9.09 %) Metastatic squamous cell carcinoma (1) (9.09 %) Tuberculosis (TB) (3) (27.27 %)	Tuberculosis (TB) (2) (18.18 %)
Non-malignant diagnosis—37 cases (62.71 %)	Tuberculosis (TB) (28) (75.67 %) TB with HIV (2) (5.40 %) Histoplasmosis (1) (2.70 %) SLE on steroids (1) (2.70 %)		
Undetermined cause—1 case (1.69 %)			1 case (1.69 %)

which bone pain was the presenting feature in majority of cases. Bony pain was seen only in 11.3 % of our cases. Our findings were very similar to other Indian studies [11–13]. This could be explained based on the extent of necrosis as well as different etiologic associations, TB being the most common cause of BMN with majority being grade I BMN in this study.

The most common peripheral blood abnormalities noted in our study were anemia (87.1 %), bicytopenia (79 %), and followed by thrombocytopenia (67.5 %). These findings were in agreement with study by Paydas et al. [10] and Rekha et al. [12] from India. Pancytopenia and abnormal cells ($p=0.000$) were seen in 32.3 and 27.4 %, respectively. Leucoerythroblastic picture (30.6 %) ($p=0.000$) should prompt one to look for underlying hematopoietic malignancy.

In contrast to most other Western studies [1, 4, 14–17], non-malignant diagnosis dominated in our study. This was because of high prevalence of TB, the most common underlying etiology for BMN accounting for 59.32 % cases in our study, even though most of these cases (81.07 %) had grade I BMN. This involvement of bone marrow as a part of disseminated TB was noted in 10 cases, similarly to a case described by Lee et al. group [18]. Most of the Indian studies had excluded TB cases from their study and had not adopted this BMN Maisel et al. [1] grading system, thus explaining the discrepancies noted in this study [11–13]. If we exclude TB cases from our study, hemato-lymphoid malignancies were seen in nearly two-third of cases and metastatic solid malignancies in remaining one-third of our cases, as expected. Most of these cases had grade II and III BMN.

In our study, an interesting case of SLE patient on long-term steroid therapy who developed pancytopenia was recorded. On BMB, grade I BMN was noted. This patient had no other necrotic-based clinical manifestations like Kikuchi's syndrome or panniculitis. No infective etiology was seen on special stains. Paydas et al. [19] reported an association of anti-phospholipid antibody (APLA) syndrome with BMN. Arterial thrombosis was responsible for BMN in such cases. However, we had evaluated this SLE case for APLA syndrome, and investigations were found to be negative.

Underlying diagnosis could not be established in one of the cases. This was a case of an eight-year-old child who presented with pancytopenia and transfusion dependent anemia. Bone marrow aspirate showed chiefly lymphocytes with necrotic background, and BMB showed >90 % BMN (grade III). Even after extensive work-up, etiology could not be identified in this case, and patient was lost to follow up.

The exact pathogenesis of BMN is unclear [4]. It was hypothesized that infiltration of nutrient blood vessels in medullary cavity by the tumor cells resulting in ischemia is the major underlying mechanism. Additional factors like chemotherapy, radiotherapy, and cytokines produced by tumor cells may also play a role [4, 16].

The prognosis of finding BMN in patients with hematolymphoid malignancy is invariably dismal. None of the patients achieved complete remission (CR) in a study by Cassileth et al. [16]. In our study, two cases of AML required second induction course to achieve CR, one case each of T-ALL and B-ALL with BMN at diagnosis succumb to death by extensive fungal infection and disease within induction phase of chemotherapy.

Our study is one of the largest series of BMN coming from developing countries. It underscores the importance of infections, particularly tuberculosis, among the different etiologies of BMN. Hematological malignancies accounted for majority of remaining cases. However, our study also suffers from the usual drawbacks of being retrospective in nature, as some of the biochemical parameters could not be incorporated.

To conclude, BMN is a rare clinico-pathological entity with a poor prognosis. Although, the etiology of BMN is varied, this differential possibility should be kept in mind in any patient presenting with bicytopenia or pancytopenia especially in the setting of infections and malignancies. Tuberculosis should always be an important differential diagnosis in developing countries like India, before considering malignancy. A good clinical examination and valuable opinion from experienced hemato-pathologist along with other ancillary studies should aid one to reach possible diagnosis in majority of BMN cases.

Conflict of interest The authors declare that they have no competing interest.

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