Case Report

New Bone Formation in Haematological Malignancies- A Novel Observation in A Series of 5 Cases

Amita Jain Gupta, Poonam Rani*, Roopal Rathi, Tejinder Singh

Department of Pathology, Maulana Azad Medical College, Delhi, India

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ABSTRACT

New bone formation in haematological malignancies is rare and its clinical significance is not known. Only very few cases of new bone formation in haematological malignancies have been described however, it is well known in metastatic prostate and breast cancer. Cases of acute megakaryocytic leukemia, primary myelofibrosis and pediatric cases of acute myeloid leukemia have also demonstrated osteosclerosis and have been associated with poor prognosis.

New bone formation is an incidental finding in bone marrow biopsy and an association with cytopenias and fibrosis has been noted. Marrow replacement by new bone aggravates the already existent cytopenias, which occur due to malignant cells replacing the normal hematopoietic precursors.

We describe two cases of acute Leukemia and three cases of Non-Hodgkin Lymphoma with new bone formation evident in bone marrow biopsy with the newly formed woven bone replacing the normal marrow elements. Pancytopenia was seen in 3 cases and bicytopenia in remaining two cases. Also fibrosis was present in two cases.

New bone formation may be found in hematological malignancies and contributes to pancytopenia and should be searched for. Medications modulating bone metabolism may be evaluated along with the chemotherapy in such patients, as they might increase the rate of remission in the hematological neoplasms. Recognition of bony changes in the marrow due to the leukemia effect per se; and not as a part of generalized bone disease, may prevent a battery of tests and further medication of the patient for bone disease.

***Corresponding author:**
Dr. Poonam Rani, C-312/B, street no -5, New Usmanpur, Delhi-110053, (INDIA)
Phone: +91 9871357125

Introduction

New bone formation and its role has been rarely reported and studied in leukemia/ lymphoma involving the bone marrow. It is a frequent finding in metastasis from prostate and breast cancer. There is lack of knowledge about its implications on the clinical course. Marrow replacement by new bone aggravates the already existent cytopenias, which occur due to malignant cells replacing the normal hematopoietic precursors. We document 5 cases of new bone formation in different hematological malignancies and discuss the implication of this finding.

Case Report(S)

5 cases of different haematological malignancies were found to have new bone formation in bone marrow biopsy, and are described below.

Case 1: A 14 year old male patient presented with complaints of fever since one month, fatigue and weight loss. Abdominal examination revealed hepatosplenomegaly (HSM) (liver 6cm and spleen 7 cm below costal margin). His hemoglobin was 5g/dl, total leucocyte count was 3000/ mm^3, and platelet count 7000/ mm^3. On peripheral smear, Red blood cells (RBC) were normocytic normochromic red cells with presence of few macrocytes and 27% blasts having scant cytoplasm, high N/C ratio and 1-2 prominent nucleoli. Bone marrow aspirate (BMA) smears were diluted with peripheral blood however, revealed similar blast cells constituting 81% of marrow nucleated cells. Cytochemistry performed on peripheral blood (PB) as well as bone marrow demonstrated block positivity for Periodic Acid Schiff stain in blasts while they were negative for myeloperoxidase stain. *Immunophenotyping* was performed on peripheral blood using flow cytometry. 20 % cells with low side scatter and dim CD 45 positivity were gated, corresponding to blast population. Out of gated cells 98% cells showed moderate CD19 and CD 10 expression.(*Image1*) The blasts were negative for cytoplasmic CD3, cytoplasmic MPO, cytoplasmic CD79a, CD 13, CD2 and CD7. A diagnosis of **CALLA positive B cell Acute Lymphoblastic Leukemia(ALL)** was given. Bone marrow biopsy showed complete replacement of marrow by blasts with marked paucity of hematopoietic elements. Also seen were tongues of woven bone invading the marrow spaces. (*Image2*)

Case 2: A 17 year old female presented with pain abdomen and fatigue. On examination patient had pallor and hepatosplenomegaly. *Hemogram* findings were hemoglobin- 6.3g/dl, total leucocyte count -6930/mm^3, platelet count - 90000/mm^3. Peripheral smears showed macrocytic blood picture with presence of 5 nucleated RBC / 100 WBC and left shift in myeloid series. Differential leucocyte count: Myelocytes 6, Metamyelocytes3, Polymorphs 59, Lymphocytes 24, Monocyte 7, Eosinophil 1 %. There were no atypical cells in the peripheral blood. But, *bone marrow aspirate* revealed 85% blasts replacing the bone marrow with near total absence of myeloid, erythroid and megakaryocytic series. Blasts had high N/C ratio, coarse nuclear chromatin and indistinct nucleoli. Blasts showed block positivity for Periodic acid Schiff and were negative for MPO on *cytochemistry*. The bone marrow was not available in EDTA so flow cytometric analysis could not be performed. *Bone marrow biopsy* picture was also replaced by blast cells with similar morphology as bone marrow aspirate.(*Image3*) *The immunohistochemical* markers were non-contributory. A diagnosis of **acute aleukemic leukemia (lymphoid)** was given. This case also showed new bone formation and was associated with grade 2 fibrosis.

Case 3: A 50 year old female presented with complaints of fever since 20 days and fatigue. Per-abdominal examination revealed hepatosplenomegaly and multiple abdominal lymph nodes. *Hemogram* showed bicytopenia (Hemoglobin of 6.2g/dl, Total Leucocyte Count: 4,350/ mm^3 and platelet count -60000/ mm^3). Red cells were normocytic normochromic with rouleaux formation. On peripheral smear, differential count was: Atypical cells-6, polymorphs-20, lymphocytes-70, Monocyte-2, Eosinophils-2%. *Bone marrow imprint* smears were hypercellular and showed sheets of atypical cells, which were large with eccentric nucleus and pale basophilic cytoplasm, open chromatin and occasional 1-2 nucleoli. There was marked paucity of hematopoietic precursors. *The bone marrow biopsy* was hypercellular. Marrow spaces were replaced by diffuse sheets of atypical lymphoid cells with coarse nuclear chromatin and moderate amount of cytoplasm. Other hematopoietic precursors were scanty in number. There was evidence of new bone formation which was laid down over endosteal surfaces of pre-existing bony trabeculae like the above cases. (*Image4*) *On immunohistochemistry* (IHC), atypical cells were immunoreactive for B cell marker (CD 19) and were negative for T cell marker, anti-myeloperoxidase. The reactive lymphocytes which comprised a small population expressed T cell marker. On the basis of morphological and immunohistochemical findings, the final diagnosis of **Non-Hodgkin lymphoma- B cell type (B-NHL)** was made.

Case 4: An eighteen year old patient presented with fever and pallor. *Hemogram* showed pancytopenia with hemoglobin- 3.2 g/dl, total leucocyte count – 1230/mm^3 and platelet count of 9000/mm^3. The differential count showed 5 % atypical cells. *Bone marrow aspirate* was a dry tap. *Bone marrow biopsy* was markedly hypercellular

and nearly half of the marrow demonstrated infiltration by monomorphic cells along with marrow fibrosis and focal necrosis. The atypical cells had vesicular nuclei with some cells showing nuclear indentation and were positive for CD19. There was frequent mitosis. Marked paucity of hematopoeitic precursors was noted. This case also showed new bone formation. (*Image5*) Patient was diagnosed as **Non-Hodgkin lymphoma, B cell type**.

Case 5: was a 28 year old male patient with weight loss, anorexia and pain abdomen since six months. The spleen was enlarged to 2 cm below costal margin. Peripheral blood examination revealed pancytopenia (Hemoglobin-4.2 g/dl, Total Leucocyte Count- 3200/mm^3 and Platelet count -30000/mm^3. The bone marrow biopsy showed fibrosis and thus aspirate was not possible. There was single cell coagulative necrosis along with few atypical hyperchromatic monomorphic lymphoid cells in a nodular deposit in the biopsy. No granuloma formation/ Reed Sternberg cell could be identified in the multiple serial sections. There was focal gelatinous marrow transformation along with normal hematopoietic precursors in rest of the marrow spaces. This case also had new bone formation in the form of tongues of osteoid arising from well-formed bony trabeculae. (*Image6*) Immunohistochemistry was noncontributory. The diagnosis of Non-Hodgkin lymphoma was suggested on the basis of morphology as in the present case the atypical lymphoid cells were present in nodular deposit rather than in diffuse pattern as seen in leukemia. The bone marrow involvement by lymphoma can be either nodular, diffuse or paratrabecular whereas in cases of acute leukemia the involvement is either paratrabecular or diffuse thus differentiating the present case from acute leukemia.

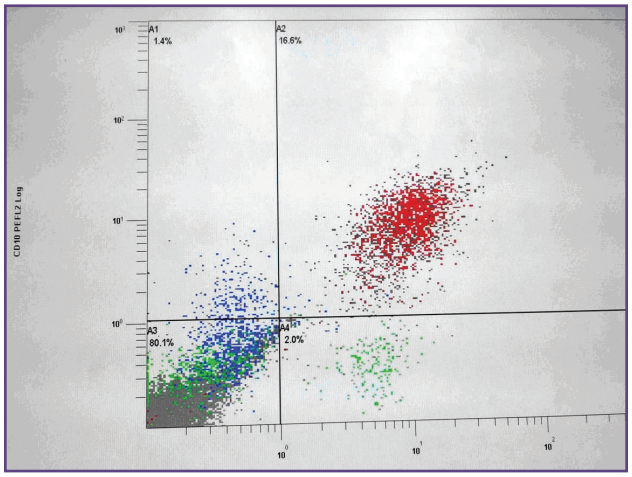

Image 1 *(Case 1):* CD10 & CD19 positive blasts on Flow cytometry.

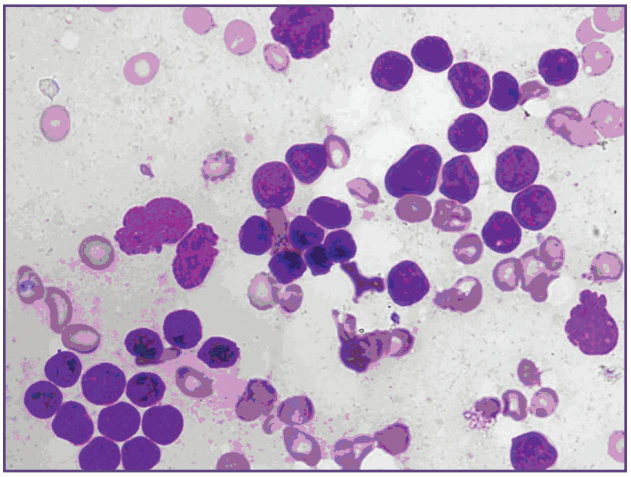

Image3 *(Case 2)*: Blasts in bone marrow aspirate. No blasts identified in peripheral blood. (Giemsa, 200X): Aleukemic leukemia

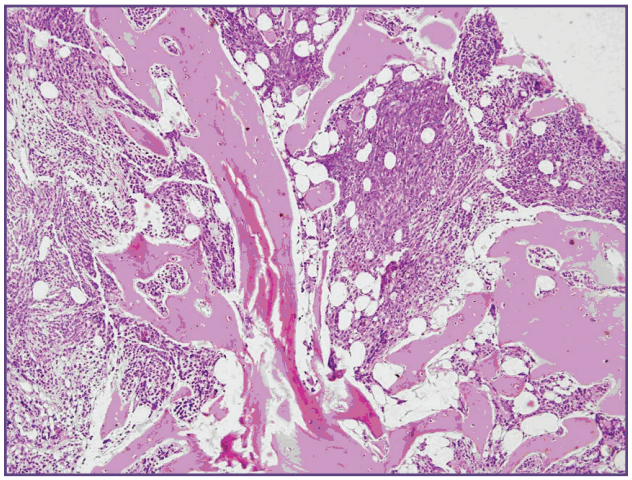

Image2 *(Case 1)*: Bone marrow biopsy shows new bone formation in case of acute lymphoblastic leukemia. (H&E, 200X)

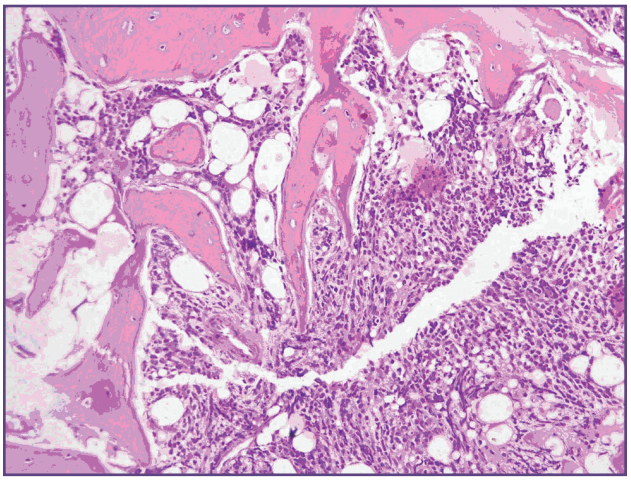

Image4 *(Case 3)*: Tongues of new bone invading the tumor cells; Non Hodgkin Lymphoma (H&E, 200X)

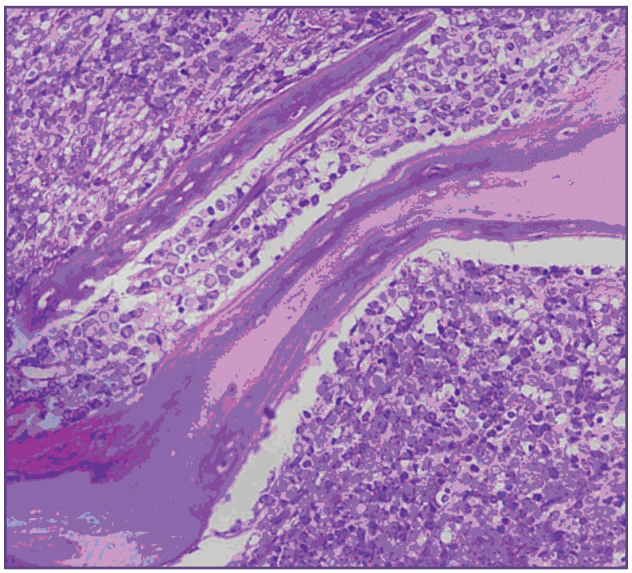

Image5 *(Case 4)*: Bone marrow biopsy showing osteogenesis in Non Hodgkin Lymphoma. (H&E, 200X)

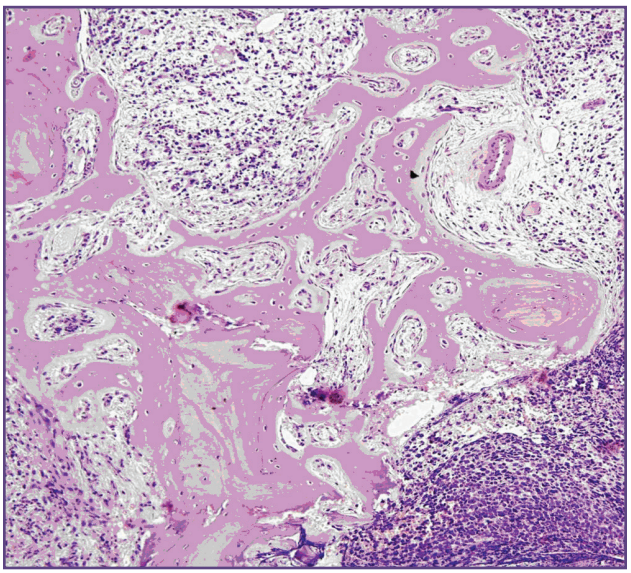

Image 6 *(Case 5)*: New bone formation along with nodular deposit of atypical lymphoid cells in Non Hodgkin Lymphoma. (H&E, 200X)

TABLE 1: Clinical and hematological findings

Case	Age (Years)	Sex	HSM	Atypical Cells In PB(%)	Atypical Cells In BMA (%)	Bone marrow biopsy	Fibrosis	Diagnosis
1	14	M	+	27	81	Replaced	_	B-ALL
2	17	F	+	0	85	Replaced	_	Aleukemic leukemia (ALL)
3	50	F	+	06	95	Replaced	_	NHL-B
4	18	M	+	05	80	Interstitial	+	NHL-B
5	28	M	+	0	Dry Tap	Nodular	+	NHL

****NOTE:** HSM: Hepatosplenomegaly, PB: Peripheral Blood, BMA: Bone Marrow Aspirate, BMB: Bone Marrow Biopsy, B-ALL: Acute Lymphoblastic Leukemia-B Cell type, NHL-B- Non Hodgkin Lymphoma, B cell type, (+): present, (-): absent

Discussion

Bone metabolism is a dynamic process with ongoing resorption and formation in a delicate balance. It is regulated by various hormones, vitamins and cytokines. The new bone formed is woven in character and is laid down on the endosteal surfaces of pre-existing trabeculae (appositional growth) or as islands of osteoid within the marrow spaces (intramedullary ossification). In bone marrow, new bone formation occurs frequently in cases of metastasis secondary to carcinoma prostrate, breast, kidney and stomach.[1]Documentation of new bone formation in the bone marrow in lymphoid hematological malignancies is sparse. However, osteosclerosis has been found in association with myeloid malignancies like acute megakaryocytic leukemia, primary myelofibrosis and pediatric cases of acute myeloid leukemia.[2,3,4]

Five cases of lymphoid malignancies described above are exclusive as new bone formation has been reported rarely in lymphoid leukaemia/lymphoma. Heinrich et al observed a large number of skeletal changes in patients with acute lymphoblastic leukemia including periosteal new bone formation, osteopenia and presence of sclerosis in the skeleton. It was observed that patients with larger number of bony lesions were symptomatic for a longer duration before they presented for chemotherapy and thus these changes need to be detected earlier. The recognition of these changes by treating doctors can lead to earlier diagnosis and initiation of therapy for leukemia.[5] New bone formation is also being implicated as a cause of cytopenias along with already known other causes (leukemic infiltration and marrow fibrosis). Majority of the cases with new bone formation are known to have fibrosis. [2,3,4] Similarly two of our 5 cases (NHL) also had marrow fibrosis.

Exact mechanism of bone formation in haematological malignancies is not clearly defined. Bone marrow stromal cells, which play a role in causation of various haematological malignancies, are postulated to have a role in new bone formation in few myeloproliferative

neoplasms. They are activated by various cytokines (PDGF, TGF-β, FGF and VEGF) released by neoplastic cells to synthesize extracellular matrix. Osteoprotegrin and Bone morphogentic protein-1 production in Acute pan-myelosis with myelofibrosis may contribute to increased osteoblastic activity resulting in osteosclerosis.[6] The study of these factors may help in development of targeted therapy for individual patients. Thus, recognition of these findings and investigation of their mechanism are of importance.

Changes in serum calcium and alkaline phosphatase (ALP) levels can occur owing to the alteration in bone metabolism due to leukaemic cells in various hematological malignancies. Their levels normalize along with restoration of normal cellularity in the marrow treatment.[3] It has been suggested that increased bone formation might lead to hypocalcaemia and hypophosphatemia and this might be induced by leukemic cells. Chemotherapy targeted for the specific leukemia along with calcitriol and etidronate sodium administered to such a patient might resolve the bony changes along with contribution in remission of leukemia.[7]

We suggest that recognition of bony changes in the marrow due to the leukemia effect per se; and not as a part of generalized bone disease, may prevent a battery of tests and further medication of the patient for bone disease.

The limitation of our study is that we were not able to follow up these patients to assess prognosis. Further studies need to be taken up for studying the effect of this novel finding on prognosis of patient.

Conclusion

New bone formation in haematological malignancies is rare and its clinical significance is not known. It leads to bone marrow replacement causing cytopenias and aggravating the already non-functional bone marrow due to leukemic infiltration of the cells and fibrosis. A work up of pretreatment and post remission serum calcium, phosphorus, alkaline phosphatase levels and CT scan needs to be done to detect the changes in skeleton. Also, these medications modulating the bone need to be tested if they can increase the rate of remission in the hematological neoplasms. More cases should be evaluated for new bone formation and to study its effect on prognosis, if any.

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Competing Interests

None

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