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Bone marrow aspiration study of megakaryocytic alterations in non myelodysplastic syndrome related thrombocytopenia

Neelima Tirumalasetti[1,*], Nagaraja Reddy Challa[2]

[1]Assistant Professor, Dept. of Pathology, [2]Assistant Professor, Dept. of General Medicine, Katuri Medical College & Hospital, Andhra Pradesh

***Corresponding Author:**
Email: nilu_medico@yahoo.co.in

Abstract

Background: Thrombocytopenia is a common hematological condition associated with variable etiological factors. Dysplastic changes of megakaryocytes in thrombocytopenia are commonly seen in myelodysplastic syndrome (MDS). However, several studies have described it's occurrence in non-myelodysplastic hematological conditions. The present study was undertaken to note the various morphological alterations in megakaryocytes (including both dysplastic and non dysplastic changes) in non MDS related thrombocytopenia.

Materials and Methods: A prospective study of 78 cases with thrombocytopenia were included in the present study. Cases of MDS were excluded from the study. Informed consent was taken from all the patients with thrombocytopenia and Bone marrow aspiration was done in all cases. Bone marrow aspiration smears were air dried and stained with Leishman stain. Bone marrow aspiration smears were examined for number and various morphological alterations. Special investigations were done in required cases for confirmation of diagnosis.

Results: Dysplastic megakaryocytes were observed in 78.6% cases of immune thrombocytopenic purpura and 41.2% cases of megaloblastic anemia. Most common dysplastic feature observed was micromegakaryocyte (32.0%) followed by multiple separate nuclei (21.7%) and hypogranular form was the least observed dysplastic feature (8.9%).

Conclusions: Dysplasia in megakaryocytes is a common finding in various non-myelodysplastic syndrome related thrombocytopenia. The mere presence of dysplastic megakaryocyte should not prompt an interpretation of myelodysplastic syndrome and should always be correlated with patient's clinical and other hematological parameters.

Introduction

Thrombocytopenia is a common hematological condition for which bone marrow aspiration is indicated.[1] Thrombocytopenia is encountered in various hematological disorders including myelodysplastic syndromes (MDS) as well as various non-myelodysplastic hematological conditions.[2]

Dyplastic features of megakaryocyte morphology include multiple separated nuclei, micromegakaryocytes (3-6 times larger than RBC with 1-2 lobes, mature nucleus and lower nuclear to cytoplasmic ratio) and hypogranular forms (with little or no granules). Non dysplastic features of megakaryocytes include immature forms (with basophilic cytoplasm, high nuclear to cytoplasmic ratio and no nuclear lobation), emperipolesis (intact hematopoietic cells within cytoplasm) cytoplasmic budding, vacuolization and bare nuclei without cytoplasm.[3]

Dysplastic changes in megakaryocytes are well known in thrombocytopenia associated with myelodysplastic syndrome (MDS). In contrast, few studies state that dysplastic changes are observed in several non MDS related cases of Thrombocytopenia. However, the prevalence of dysplastic features in non MDS related thrombocytopenia is still debatable. The present study was undertaken for understanding the dysplastic megakaryocytic alterations and their contribution to thrombocytopenia in non-MDS diseases so as to increase the diagnostic accuracy.

Materials and Methods

A prospective study of 78 bone marrow aspirations in patients with thrombocytopenia was included in the present study. All the cases of thrombocytopenia which were diagnosed on hematology analyzer (Platelet count <1, 50,000/cu.mm); confirmed subsequently by peripheral blood smear examination were taken up for the present study. Cases showing evidence of MDS, pseudo-thrombocytopenia or receiving chemo/radiotherapy were excluded from the study. A prior informed consent was taken from all the patients and bone marrow aspiration was done from posterior superior iliac spine/ sternum under aseptic precautions. Smears were air dried and stained with Leishman stain.

Bone marrow aspiration smears were examined for total number of megakarycoytes and morphological alterations (both dysplastic and non-dysplastic features). Megakaryocyte number was assessed by counting the smears in 10 low power fields (LPFs) and were categorized as absent, if 0 megakaryocytes are seen/10LPFs, decreased if 1/5-10LPFs, normal if 1/1-3LPFs and increased if >2/LPF.[3] At least 30 megakaryocytes were evaluated for megakaryocytic alterations including both dysplastic features (multiple separate nuclei, micromegakaryocyte and hypogranular forms) and non-dysplastic features (emperipolesis, immature, bare nuclei, cytoplasmic vacuolization and budding). The criteria to establish a case as having dysplastic features was considered when >10% of

megakaryocytes showed the above mentioned dysplastic features.[4]

Bone marrow aspiration diagnosis was correlated with peripheral smear study, clinical examination and confirmed by biochemical assays (like Serum B12, folate, iron, ferritin and TIBC) along with microbiological (viral markers like [human immunodeficiency virus (HIV), Hepatitis C virus[HCV]), radiological (Computed Tomography [CT] scan), cytochemical (Myeloperoxidase[MPO], Sudan Black B [SBB], Periodic acid Schiff stain[PAS]) and cytogenetic studies [like bcr-abl fusion gene, t(15;17) and t(8;21)]. Bone marrow biopsy was done in required patients.

Results

In the present study, out of the 78 patients with thrombocytopenia, 46 (58.9%) patients were males and 32 (41.1%) patients were females. The most common age group was between 20-39yrs (52.5%; 41patients).

Table 1 shows common causes of thrombocytopenia. Most common cause of thrombocytopenia in the present study was Megaloblastic anemia followed by Immune thrombocytopenic purpura (ITP). Special investigations were done to confirm the diagnosis and are depicted in Table 1.

Table 1: Causes of thrombocytopenia diagnosed on BMA along with special investigations

Bone marrow aspiration (BMA) Impression	No. & Percentage of Patients	Special investigations for confirmation of BMA Diagnosis
Megaloblastic anemia	34 (43.5%)	Serum B12 and Folic acid assays
ITP	14 (17.9%)	By Exclusion of causes of secondary thrombocytopenia
Infection associated thrombocytopenia (IAT)	06 (07.7%)	Viral marker study (HIV, HCV)
Hypersplenism	05 (06.5%)	Peripheral smear examination and CT abdomen
Dimorphic anemia	04 (05.1%)	Serum ferritin, iron, TIBC, B12 and Folic acid assays
Multiple Myeloma	04 (05.1%)	Bone marrow biopsy
Acute leukemia	04 (05.1%)	Cytochemistry and cytogenetics
Chronic leukemia (Blast crisis)	02 (02.6%)	Cytogenetics(bcr-abl fusion gene), NAP score
Iron Deficiency anemia	02 (02.6%)	Serum ferritin, iron and TIBC assays
Aplastic anemia	02 (02.6%)	Bone marrow biopsy
Idiopathic hypereosinophilia	01 (01.3%)	Clinical history, peripheral smear examination

Number of megakaryocytes/10LPF was noted in each patient. Patients with megaloblastic anemia and ITP had increased number of megakaryocytes. Table 2 shows number of megakaryocytes per 10 LPF's in various etiologies of bone marrow aspiration.

Table 2 Number of Megakaryocytes per 10 LPF's in various etiologies

Bone marrow aspiration (BMA) Impression	Normal	Increased	Decreased	Absent
Megaloblastic anemia	11 (32.4%)	21 (61.8%)	02 (5.8%)	-
ITP	02 (14.3%)	12 (85.7%)	-	-
Infection associated thrombocytopenia (IAT)	04 (66.6%)	01 (16.7%)	01 (16.7%)	-
Hypersplenism	-	05 (100%)	-	-
Dimorphic anemia	02 (50%)	02 (50%)	-	-
Multiple Myeloma	01 (25%)	-	03 (75%)	-
Acute leukemia	01 (25%)	01 (25%)	02 (50%)	-
Chronic leukemia (Blast crisis)	-	-	02 (100%)	-
Iron Deficiency anemia	01 (50%)	01 (50%)	-	-
Aplastic anemia	-	-	01 (50%)	01 (50%)
Idiopathic hypereosinophilia	01 (100%)	-	Nil	Nil

Dyplastic megakaryocytes were commonly seen in ITP and megaloblastic anemia depicted in Table 3.

Table 3: Prevalence of dysplastic and non-dysplastic changes in various hematological conditions

Bone marrow impression	Dysplastic changes	Non dysplastic changes	Total
Megaloblastic anemia	14 (41.2%)	20 (58.8%)	34
ITP	11 (78.6%)	03 (21.4%)	14
Infection associated thrombocytopenia (IAT)	01 (16.7%)	05 (83.3%)	06
Hypersplenism	01 (20%)	04 (80%)	05
Dimorphic anemia	02 (50%)	01 (50%)	04
Multiple Myeloma	02 (50%)	02 (50%)	04
Acute leukemia	02 (50%)	02 (50%)	04

Chronic leukemia(Blast crisis)	01 (50%)	01 (50%)	02
Iron Deficiency anemia	-	02 (100%)	02
Aplastic anemia	02 (100%)	-	02
Idiopathic hypereosinophilia	-	01 (100%)	01

Most common dysplastic feature observed was micromegakaryocyte in 25 cases (32.0%), multiple separate nuclei in 17cases (21.7%) and hypogranular form was the least observed dysplastic feature in 7cases (8.9%). Various morphological features are depicted in Table 4, Fig. 1 and Fig. 2.

Table 4: Morphological alterations of megakaryocytes in various conditions

Bone marrow impression	Immature megakaryocytes	Bare megakaryocytic nuclei	Emperipolesis	Cytoplasmic vacuolization	Budding	Multiple separate nuclei	Micromegakaryocytes	Hypogranular megakaryocytes	Hypolobation	Hyperlobation
Megaloblastic anemia	16 (47.1%	03 (8.8%	-	02 (5.9%)	-	09 (26.4 %)	10 (55.8%)	01 (2.9%)	07 (20.6%)	02 (5.9%)
ITP	10 (71.4%)	11 (78.6%)	05 (35.7%)	04 (28.6%)	01 (7.1 %)	06 (42.9 %)	08 (57.1%)	01 (7.1%)	06 (42.9%)	01 (7.1%)
Infection associated thrombocytopenia(IAT)	04 (66.7%	-	-	03 (50%)	-	-	-	-	01 (16.7%)	-
Hypersplenism	-	-	02 (40%)	01 (20%)	01 (20%	-	-	01(20%)	-	-
Dimorphic anemia	01 (25%)	01 (25%)	-	02 (50%)	01 (25%)	-	01 (25%)	2(50%)	01 (25%)	-
Multiple Myeloma	01 (25%)	01 (25%)		01 (25%)			02 (50%)			
Acute leukemia	01 (25%)	02 (50%)	02 (50%)	-	-	02 (50%)	02 (50%)	-	-	-
Chronic leukemia(Blast crisis)	01 (50%)	01 (50%)	-	-	01 (50%)		01 (50%)	01(50%)	01 (50%)	-
Iron Deficiency anemia	-	-	-	-	-	-	-	-	01 (50%)	01 (50%)
Aplastic anemia	01 (50%)	-	-	-	-	-	01 (50%)	01(50%)	01 (50%)	-
Idiopathic hypereosinophilia	01 (100%)	-	-	-	-	-	-	-	-	-

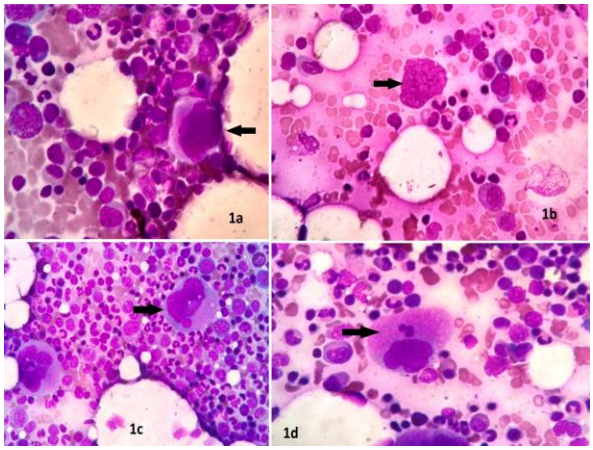

Fig. 1: Non dysplastic Morphological features of megakarycoytes

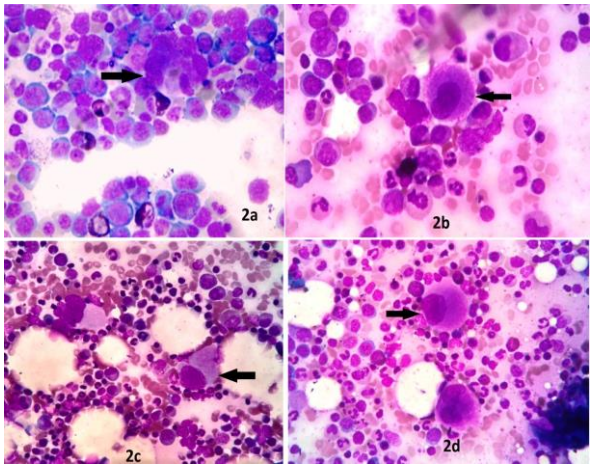

Fig. 2: Dysplastic morphological features of Megakarycoytes

Discussion

Thrombocytopenia, either persistent, isolated or in association with pancytopenia refractory to treatment is one of the commonly encountered hematological problems for which a bone marrow study is indicated. The routinely prepared Leishman stained bone marrow aspirate smears can help to observe the dysplastic features of the megakaryocytes associated with the non-MDS conditions.[5]

In the present study, out of the 78 patients with thrombocytopenia, 46 (58.9%) patients were males and 32 (41.1%) patients were females with a male is female ratio of 1.4:1; similar to studies conducted by Choudhary et al and Parul Gupta et al.[1,6] Thrombocytopenia was common in the age group between 20-39yrs (52.5% patients), as in a study by Choudhary et al.[1]

The most common cause of thrombocytopenia in the present study was megaloblastic anemia similar to study by Choudhary et al[1] followed by ITP. According to the study of Muhury M et al the most common cause was Acute Myeloid Leukemia (AML), followed by ITP & Acute Lymphoblastic Leukemia(ALL).[3] In contrast, a study conducted by Parul Gupta et al states that the most common cause of thrombocytopenia was ITP followed by megaloblastic anemia and iron deficiency anemia.[6]

Increased number of megakaryocytes in bone marrow aspiration smears were observed in 85.7% cases of ITP and 61.8% cases of megaloblastic anemia, similar to the observations of Choudhary et al & Muhury M et al.[1,6]

In the present study dyplastic megakarycoytes were found predominantly in ITP and megaloblastic anemia. Dyplastic changes in megakaryocytes were observed in 78.6% cases of ITP and 41.2% of cases of megaloblastic anemia in the present study.

In the present study, the common dysplastic feature in megakaryocytes of ITP observed was micromegakaryocytes (57.1% cases) similar to observations made by Shi Xd et al.[7] In contrast, Parul Gupta et al observes that the most common morphological alteration found in cases of ITP were hypolobulation & hypogranular forms.

In the present study, the common dysplastic feature in megakaryocytes of Megaloblastic anemia observed was micromegakaryocytes (55.8% cases) followed by multiple separate nuclei (26.4%cases). According to a study by Parul Gupta et al, the most common dysplastic changes were hypogranular forms & micromegakaryocytes; in contrast to Choudhary et al & Muhury M et al who observed multiple separate nuclei to be the most common dysplastic feature.[1,3,6]

Immature megakaryocytes were observed in four cases of IAT (66.7%) similar to studies by Meindersma and de Vries who opined that this was due to the increased megakaryocyte turn over[8]. Cytoplasmic vacuolization seen in three cases (50%), correlated with that of Chanarin and Walford and Chesney et al. These authors consider that the possible cause of cytoplasmic vacuolization could be due to toxic injury by acquired cytomegalovirus infection.[9,10] Recent studies have shown immune-mediated platelet destruction to be the cause of thrombocytopenia in human immunodeficiency virus(HIV), Hepatitis C virus and Helicobacter pylori infections.[11] Four cases in the present study had HIV infection and two cases had HCV infection.

In the present study, 100% patients with hypersplenism showed increase in megakarycoyte number. The increased number of megakaryocytes observed in all cases of hypersplenism is compensatory and can be due to removal of platelets by increased pooling and by increased phagocytosis in the spleen.[2]

Out of four cases of dimorphic anemia, dysplastic changes were observed in 50% cases; the most common being hypogranular forms & micromegakaryocytes, similar to that observed by Tejinder Singh et al.[13]

Two of the four cases of Multiple myeloma showed dysplastic changes with micromegakaryocytes

being the predominant population, similar to study by Muhury et al.[3]

Acute leukemia patients show dysplastic megakaryocytes, most common being micromegakaryocyte and multiple separate nuclei in 50% of cases, similar to studies by Choudhary et al and Frenkel et al.[1,14] In contrast, dysplastic changes in megakaryocytes were relatively less as in a study conducted by Muhury et al and the authors found dyplastic features in only 26.6% patients.[3] In a study done by Jinnai et al, dysplastic megakaryocyte (micromegakaryocyte and multiple separate nuclei) was found in only 10% of the cases of AML. They also found significantly lower response to chemotherapy in AML cases with dysplastic megakaryocytes.[15]

Out of 2 cases of CML (blast crisis), 50% showed normal number of megakaryocytes and 50% showed reduced number. Both the cases showed dysplastic features most commonly micromegakaryocytes & hypogranular forms in 50% of cases. All these findings were similar to those observed by Parul Gupta et al and Tejinder Singh et al.[6,13]

Two cases of iron deficiency anemia included in the study showed no dysplastic changes, similar to study by Choudhary et al.[1]

Out of two cases of aplastic anemia in the present study, dysplastic megakaryocytes were found in the form of micromegakaryocytes and hypogranular forms. Choudhary et al shared similar findings as the present study. In contrast, Tricot et al observed a total normal morphology in all cases of thrombocytopenia due to aplastic anemia.[1,16]

One cases of idiopathic hypereosinophilic syndrome, included in the study showed no dysplastic changes, similar to study by Choudhary et al.[1]

Hence, the present study shows that dysplastic changes in megakaryocytes were also found in non-MDS related thrombocytopenia and dysplastic morphology in megakaryocytes by themselves do not specify MDS. The observed megakaryocytic alterations may be useful in making a differential diagnosis of various etiologies of non MDS related thrombocytopenia.

Conclusions

The most common cause of thrombocytopenia in the present study was Megaloblastic anemia followed by Immune thrombocytopenic purpura (ITP). Dyplastic megakaryocytes were commonly seen in ITP and megaloblastic anemia. Hypogranular forms were the least common dysplastic morphology observed in this study and should be looked for in a suspected case of MDS. Dysplastic features in megakaryocytes were observed in various etiologies of non MDS related thrombocytopenia. Hence the cut-off value of dysplastic feature categorization should be >10%. Further comparative study with increased sample size including cases of MDS should be done to understand the occurrence of dysplastic megakaryocytes in various non-MDS related thrombocytopenias.

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