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Morphological Pattern Analysis of Megakaryocytes Inthrombocytopenia of Varied Causes

[1]Dr.Supriya Papaiah, Assistant Professor, Department of Pathology, Yenepoya Medical College, Yenepoya Deemed to be University, Deralakatte, Mangalore, Karanataka, India.

[2]Dr Nisha Thattamparambil Gopalakrishnan, Associate Professor, Department of Pathology, MES Medical College, Perinthalmanna, Malappuram, Kerala, India.

Corresponding Author: Dr Nisha Thattamparambil Gopalakrishnan, Associate Professor, Department of Pathology, MES Medical College, Perinthalmanna, Malappuram, Kerala, India.

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Abstract

Introduction: Thrombocytopenia is defined as platelet count less than 1,50,000/mm ^{3} and commonly encountered in haematological disorders ranging from benign to malignant conditions. Dysplastic features are well known in myelodysplastic syndrome (MDS) and also observed in non MDS haematological conditions.The present study was conducted to study the prevalence of various conditions associated with thrombocytopenia and to evaluate the various megakaryocytic alterations in the bone marrow aspirations in both non-MDS and MDS related thrombocytopenia cases.

Methods: A total of 60 cases of thrombocytopenia were retrospectively studied over a period of one year and bone marrow aspiration smears were analysed for megakaryocyte number and various morphological alterations. All bone marrow aspirations in this study period were retrieved and slides were reviewed. The clinical details and diagnosis were also noted.

Results: In the 60 cases thus analysed, megakaryocyte number was increased in 38 cases (63.3%), normal in 17 cases (28.3%) and decreased in 5 cases (8.3%). The most common cause of thrombocytopenia was Pancytopenia (38.3%) followed by Megaloblastic anaemia (15%) and Hypersplenism (11.6%).Bare megakaryocytic nuclei (85%) and hypolobated megakaryocytes (83.3%) were the common morphological changes in megakaryocytes. All the cases showed both non dysplastic and dysplastic features. Most common dysplastic feature observed was micromegakaryocyte (68.3%) followed by multiple separate nuclei (16.6%). Micromegakaryocyte was the most common dysplastic feature seen in 74% cases of pancytopenia, 77.7% cases of megaloblastic anemia and

71.4% cases of hypersplenism. Similarly bare megakaryocytic nucleiwas the most common non dysplastic feature seen in 91.3% of pancytopenia, 88.8% of megaloblastic anemia and 85% cases of hypersplenism.

Conclusion: Dysplastic megakaryocytes being a common finding in various non-MDS related thrombocytopenia cases, it's presence should not prompt an interpretation of MDS and should always be correlated with patient's clinical and other hematological parameters.

K**eywords:** Thrombocytopenia, Bone Marrow, Megakaryocytes, morphology

Introduction

Megakaryocytes arise from pluripotent hematopoietic stem cells that undergo differentiation and proliferation under the influence of cytokines, particularly thrombopoietin (TPO) and growth factors like hematopoietic cytokines and transcriptional factors.[1,2,3] Endoreduplication and expansion of cytoplasmic mass are the indices of maturation of megakaryocytes. Platelets are formed from the cytoplasmic buddings of megakaryocytes and results from megakaryocytic deoxyribonucleic acid (DNA) replication forming a large lobulated, polypoid nucleus. [1,2,3,4,5] Through different steps of maturation and remodeling a single megakaryocyte releases about thousands of platelets[1,2] Any abnormality or defect in the process of remodeling results in clinically significant disorders[1]. Platelets play an important role in wound repair, reduces the risk of bleeding and also maintains vascular damages by providing adequate clot formation.[1] The average platelet count ranges from 150,000 – 350,0000/μL.[1,2,5]Thrombocytopenia is defined as platelet count less than 1,50,000/μL and is commonly found in many non haematological and haematological conditions ranging from benign to malignant lesions.[1,2,3,5,6] Thrombocytopenia can result due deficient platelet production, abnormal distribution or pooling of the platelets within the body, accelerated platelet destruction, or may be artifactual.[1,7,8]As megakaryocyte morphology plays an important role in thrombopoiesis, any defect in megakaryocytopoiesis can result in dysmegakaryocytopoiesis and thrombocytopenia.[1] Dyplastic features of megakaryocyte morphology include micromegakaryocytes, multiple separated nuclei and hypogranular form with little or no granules. The non dysplastic megakaryocytic features are immature forms with high N:C ratio, basophilic cytoplasm without nuclear lobation, cytoplasmic budding, cytoplasmic vacuolization, bare nuclei without cytoplasm and emperipolesis which shows intact hematopoietic cells within cytoplasm.[1,3,5,6] Thrombocytopenia and its associated dysplastic megakaryocytic alterations are commonly seen in both Myelodysplastic Syndromes (MDS) and non-myelodysplastic hematological conditions like infection associated thrombocytopenia (IAT), like immune thrombocytopenic purpura (ITP), aplastic anemia (AA), hypersplenism, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), bone marrow metastasis and blast crisis of chronic myeloid leukemia.[1,2,3,6]

The aim of this study was to study various etiological conditions causing thrombocytopenia and to analyse the megakaryocytic alterations associated with thrombocytopenia and also to evaluate any significant association of dysplastic features in cases of thrombocytopenia.

Material And Methods

A total of 60 bone marrow aspiration smears of thrombocytopenia cases were analysed retrospectively over a period of one year.Bone marrow aspiration smears were collected from archives as well as the clinical details, complete blood counts and other relevant laboratory investigations were obtained from the medical record section of all diagnosed cases of thrombocytopenia for which bone marrow aspiration was done. The bone marrow smears were air dried and stained with Leishman stain. Exclusion criteria included cases receiving chemotherapy/radiotherapy or cases of pseudo thrombocytopenia.

The aspiration smears were analysed formegakaryocyte number and morphological alteration of megakaryocytes and cases were tabulated accordingly. The number of megakaryocytes were rated as normal(1 megakaryocyte/1-3 Low Power Field), increased (greater than 2 megakaryocytes/low power field), decreased (1 megakaryocytes/5-10).[1,3,4] A minimum of 30 megakaryocytes were assessed to look for megakaryocytic morphological changes like dysplastic forms, micro-megakaryocytes, hypo granular forms, immature forms, nuclear segmentation, platelet budding, emperipolesis, cytoplasmic vacuolations, and bare megakaryocytes. Megakaryocytes with four to sixteen nuclear lobes were considered normal. Dysplastic megakaryocytes include megakaryocytes with multiple separated nuclei, micro-megakaryocytes and hypo granularforms.[1,2,3,4,7] Non dysplastic features include immature forms, emperipolesis, cytoplasmic vacuolations, and bare nuclei without cytoplasm.[5,6,7] The number and morphology of the megakaryocytes in thrombocytopenia was then assessed. Collected data were checked for completeness and entered into Microsoft excel sheet. Descriptive statistics in terms of frequency tables, pie charts, bar diagrams were prepared.

Results

In the 60 cases thus analyzed megakaryocyte number was increased in 38 cases (63.3%), normal in 17 cases (28.3%) and decreased in 5 cases (8.3%) (Fig1). The most common cause of thrombocytopenia in our study was Pancytopenia seen in 23 cases, followed by megaloblastic anemia (9), Hypersplenism (7), Myelodysplastic syndrome (4), Hemophagocytic lymphohistiocytosis (3), Nutritional anemia (2), Acute myeloid leukemia (4), Anemia of chronic disease(2), Fanconi's anemia (2), Myelofibrosis (2) and Infection associated thrombocytopenia (2) (Chart 1).

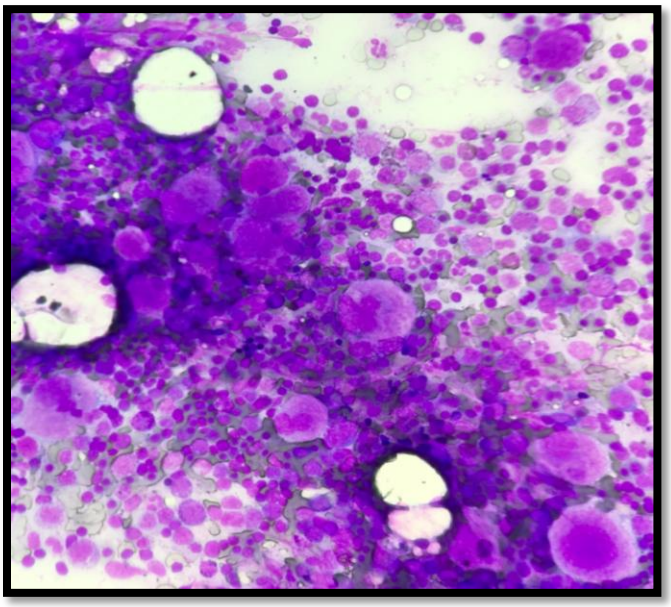

Fig 1: Increased number of megakaryocytes ((Leishman, 4X)

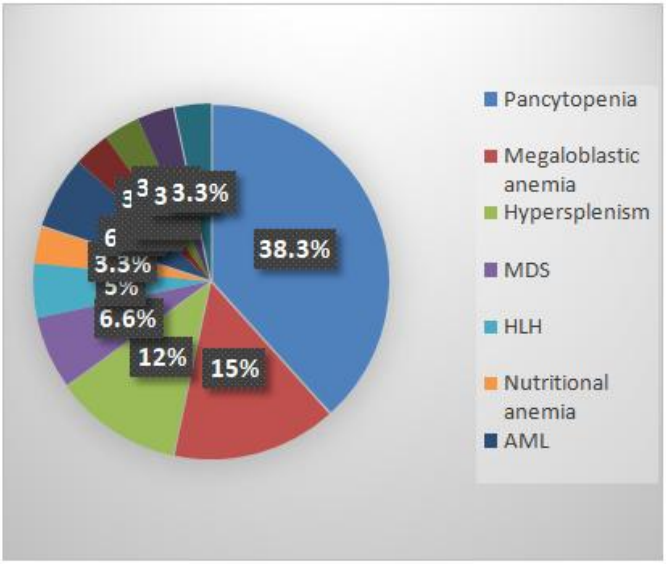

Chart 1: Showing etiology wise distribution of patients with thrombocytopenia

Bare megakaryocytic nuclei (85%) and hypolobated megakaryocytes (83.3%) were the common morphological changes in megakaryocytes (Fig2). All the cases showed both non dysplastic and dysplastic features.Fig 3 shows different morphological changes observed in megakaryocytes in our study. Most common dysplastic feature observed was micromegakaryocyte (68.3%) followed by multiple separate nuclei (16.6%) (Fig 3,4). Micromegakaryocytes was the most common dysplastic feature seen in 73.9% cases of pancytopenia, 77.7% cases of megaloblastic anemia and 71.4% cases of hypersplenism. Similarly bare megakaryocytic nuclei was the most common non dysplastic feature seen in 91.3% of pancytopenia, 88.8% of megaloblastic anemia and 85% cases of hypersplenism (Fig 2). Table1 shows various morphological alterations of megakaryocytes in various conditions of thrombocytopenia.

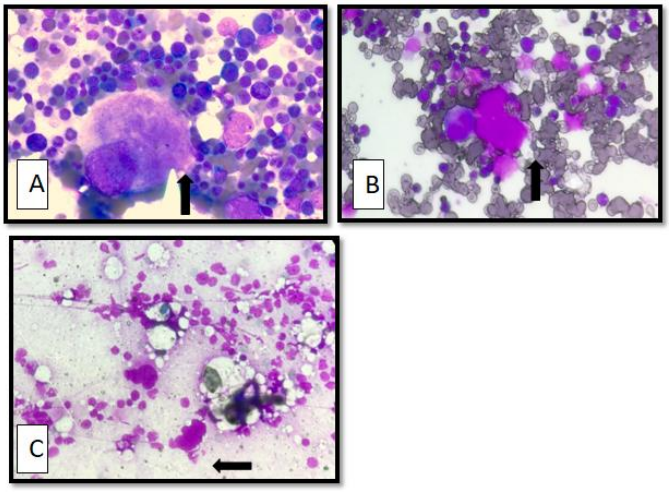

Figure 2: BM aspiration smear showing (A) Hypolobated megakaryocytes
(B),(C) Bare megakaryocytic nuclei. (Leishman stain, 40x)

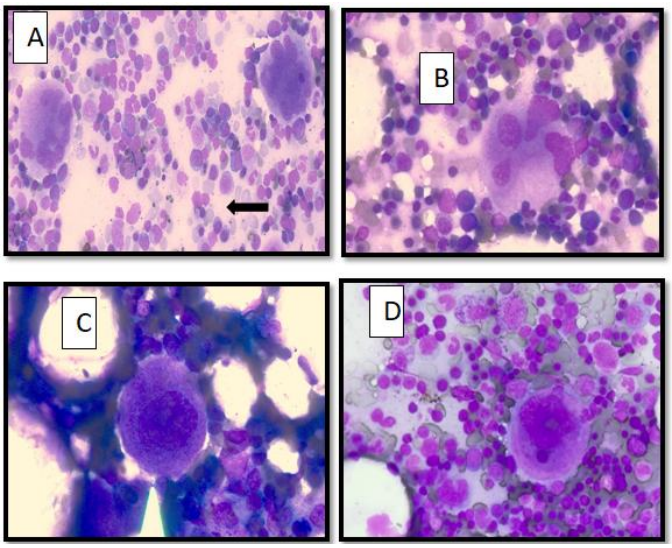

Figure 3: BM aspiration smear showing (A) Hyperlobated megakaryocytes, (B) multiple seperate nuclei,(C) Immature megakaryocytes, (D) Emperipolesis (Leishman stain, 40x)

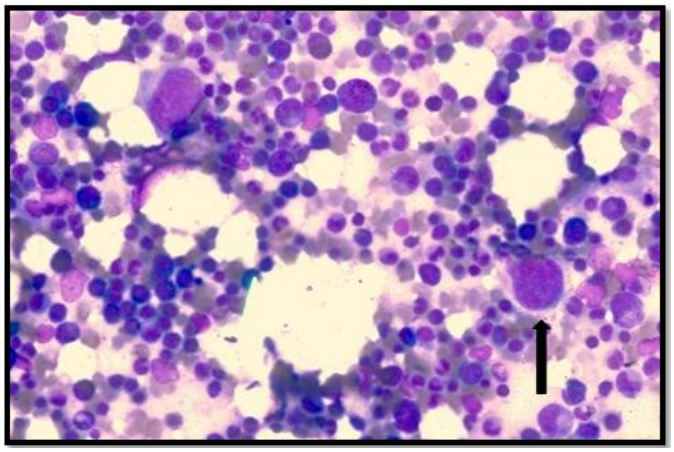

Figure 4. BM aspiration smear showing Micromegakaryocyte. (Leishman stain, 40x)

Table 1: showing morphological alterations of megakaryocytes in various conditions

Bone marrow impression	Dysplastic features			Non dysplastic features				
	Micro-mgk	Multiple separate nuclei	Hypogranular	Immature forms	Emperipolesis	Cytoplasmic budding	Bare nuclei	Hyperlobated	Hypolobated
Pancytopenia	17 (73.9%)	3 (13%)		14 (60.8%)	3 (13%)	2 (8.6%)	21 (91.3%)	1 (4.3%)	18 (78.2%)
Megaloblastic anemia	7(77.7%)	1 (11.1%)	1(11.1%)	5 (55.5%)	1 (4.3%)	1 (11.1%)	8 (88.8%)	2 (22.2%)	7 (77.7%)
Hypersplenism	5 (71.4%)	2 (28.5%)		2 (28.5%)	1 (14.2%)		6 (85%)		7(100%)
Mds	3 (75%)	3 (75%)		2 (50%)			4 (100%)		4 (100%)
Hlh	3 (100%)	1 (33.3%)		3 (100%)			3 (100%)		3 (100%)
Na				1(50%)			2 (100%)		2 (100%)
Aml	2 (50%)			2 (50%)	1 (25%)		1 (25%)		2 (50%)
Acd							2 (100%)		2 (100%)
Fa	1 (50%)						2 (100%)		1 (50%)
Mf	1 (50%)			2 (100%)			1(50%)		2 (100%)
Iat	2 (100%)			1 (50%)			1 (50%)		2 (100%)
Total	41(68.3%)	10 (16.6%)	1	32	6	3	51 (85%)	3	50 (83.3%)

Discussion

Thrombocytopenia associated with pancytopenia is a commonly encountered hematological problem and most common indication for bone marrow aspiration study. The normal maturation of megakaryocytes includes a single polypoid nucleus in which DNA keeps on multiplying itself without cytoplasmic division and after full maturation, they deform and spread out their internal membrane to form long extensions and eventually platelets are shed out. Dysmegakaryopoiesis and thrombocytopenia mainly results due to the defect in the stages of megakaryopoiesis.[3,4] Leishman-stained bone marrow aspirate smears helps to identify the number, morphologic features and dysplastic features of the

megakaryocytes associated with different cases of thrombocytopenia. This improves the diagnostic accuracy for a wide range of hematological disorders thereby enabling proper therapeutic interventions.[1,5,6]

In the present study, pancytopenia was the commonest cause of thrombocytopenia, similar observation was made in study done by Sharma R et al.[3] However the commonest cause of thrombocytopenia was AML in study done by Pokharel et al (27/144 cases, 18.8%) and MuhuryM et al.[1,5] In the studies done by Neelima et al and Choudary et al found that megaloblastic anemia followed by ITP was the common cause.[6,9] Parul gupta et al found that ITP followed by megaloblastic anemia and iron deficiency anemia were the most common causes.[10]

In the present study megakaryocyte number was increased in 38 cases (63.3%), normal in 17 cases (28.3%) and decreased in 5 cases (8.3%). In the study by Pokharel et al, 15 of the 17 cases showed increased in the number of megakaryocytes which was also observed by George et al and Levine et al. They attributed this to stimulation of the marrow megakaryocytes to synthesize platelets at an increased rate due to immune-mediated platelet destruction in the spleen and other reticuloendothelial tissues.[1,11,12,13,14,15]Neelima et al observed that increased number of megakaryocytes were seen in 85.7% cases of ITP and 61.8% cases of megaloblastic anemia, similar observations were also made by Choudhary et al &Muhury M et al.[5,6,9]

Bare megakaryocytic nuclei (51 (85%)) and hypolobated megakaryocytes (50 (83.3%)) were commonest morphological change seen in our study whereas study done by Neelima et al andMuhury m et al found that immature megakaryocyte in 46.1% and 40.2% of the cases respectively.[5,6] Parul gupta et al found hypolobated megakaryocyte (53%) as commonest morphological change.[10]

In our study the common non dysplastic feature was Bare megakaryocytic nuclei seen in 91.3% of pancytopenia, 88.8% of megaloblastic anemia (MA), whereas immature megakaryocyte was seen as the commonest non dysplastic feature in 42%, 46.1% and 40.2% in studies done by Sharma R et al, Neelima et al and Muhury et al respectively.[3,5,6]

Micromegakaryocyte (68.3%), was the common dysplastic feature in our study seen in 74% of pancytopenia and 77.7% of MA, similar observations were seen in study done by Neelima et al who found in 57.1% of ITP and 55.8% of MA cases. Whereas hypogranular megakaryocyte was commonest dysplastic feature in 58.3% of MA and 35.2% of ITP cases in study done by Parul guptaet al.[6.10]

Hence, the present study shows that dysplastic changes in megakaryocytes were also found in non MDS related thrombocytopenia like pancytopenia, Megaloblastic anemia, hypersplenism,HLH, Acute myeloid leukemia, Infection associated 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.

Conclusion

Proper evaluation of the number and morphology of megakaryocytes, during reporting of bone marrow aspirate study plays an important role in diagnostic accuracy of various hematological conditions and also provides better understanding of the pathogenesis of various hematological conditions with

thrombocytopenia, thereby enabling proper therapeutic interventions.

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