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Study of Megakaryocytes in Bone Marrow Aspiration Smears in Patients with Thrombocytopenia

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Abstract:
Background*: Dysplastic changes in megakaryocyte are well-recognized features of myelodysplastic syndrome. This study was done to understand the various megakaryocytic alterations including the dysplastic forms in hematological disorders presenting with thrombocytopenia other than in myelodysplastic syndrome.*
Materials and Methods*: A prospective study of 50 bone marrow aspirations in patients with thrombocytopenia was conducted for a period of 1 year from June,2014 to May,2015 in the Department Of Pathology, Government Medical College, Bhavnagar, Gujarat to assess the number & morphology of the megakaryocytes in non-MDS related thrombocytopenia.*
Results*: Dysplastic features were observed in 100%, 83.3% & 75% of the cases of dimorphic anaemia, iron deficiency anaemia & aplastic anaemia respectively. Most common dysplastic feature observed were hypolobulation & hypogranular forms.*
Conclusion: *The mere presence of dysplastic megakaryocyte should not prompt an interpretation of myelodysplastic syndromes and should always be correlated with patient's clinical and other hematological parameters.*
Keywords: *Megakaryocytes, Thrombocytopenia, Bone marrow, Non-myelodysplastic syndrome diseases.*

I. Introduction

Platelets are formed and released into the bloodstream by precursor cells called megakaryocytes (MK) that are derived from haematopoietic stem cells (HSCs) under the influence of Thrombopoietin (TPO), which evolve from the multipotential haemangioblast.Mature MKs give rise to circulating platelets by the acquisition of the cytoplasmic structural and functional characteristics necessary for platelet action. The cells in megakaryocytic series are least in number but largest of all haematopoietic cells. Endoreduplication (polyploidisation) and expansion of cytoplasmic mass are the hallmarks of MK maturation. The production of platelets by megakaryocytes requires an intricate series of remodeling events that result in the release of thousands of platelets from a single megakaryocyte. The stages of maturation are Megakaryoblast, Promegakaryocyte, Granular megakaryocyte, Mature megakaryocyte & Platelets. Abnormalities in this process can result in Dysmegakaryocytopoiesis which in turn would result in clinically significant disorders. A diversity of factors can contribute to anomalous platelet counts; one of these is inappropriate platelet production. Thrombocytopenia (platelet counts less than 150,000/μl) can lead to inadequate clot formation and increased risk of bleeding.[5]

Thrombocytopenia is a very common haematological condition for which a bone marrow aspiration is sought. Immune thrombocytopenic purpura (ITP) is a very common cause of thrombocytopenia. Various other haematological conditions such as megaloblastic anemia, aplastic anemia and leukemias can also present with thrombocytopenia.[1]

Dysmegakaryocytopoiesis is characterized by various megakaryocytic alterations in bone marrow aspirates and include both dysplastic and non-dysplastic features. Dysplastic features of megakaryocytes are multiple separated nuclei, micromegakaryocytes (size of large lymphocyte/monocyte with single or bilobed nucleus) and hypogranular forms (megakaryocyte with pale grey or water clear cytoplasm and sparse or no granules).Non-dysplastic features include immature forms, emperipolesis, budding, cytoplasmic vacuolization and bare nuclei. Immature megakaryocytes are young forms with scant bluish cytoplasm and lack lobulation. Megakaryocytes are considered to show budding if there is blebbing of cytoplasm on their surface. Emperipolesis has been defined by the presence of other cell within the cytoplasm of megakaryocyte.[1]

Dysplastic megakaryocytic alterations are well known in thrombocytopenia associated with myelodysplastic syndrome (MDS). However, several studies have described it's occurrence in non-myelodysplastic haematological conditions but scant data exist on their prevalence.[1]

The study was undertaken for better understanding of the dysplastic megakaryocytic alterations and their contribution to thrombocytopenia in non-MDS diseases so as to increase the diagnostic accuracy.[1]

II. Material And Methods

A prospective study of 50 bone marrow aspirations in patients with thrombocytopenia was conducted for a period of 1 year from June,2014 to May,2015in the Department Of Pathology, Government Medical College, Bhavnagar, Gujarat. All the cases of thrombocytopenia which were diagnosed on haematology analyser (platelet count <1,50,000); confirmed subsequently by peripheral smear were taken up for the study. Bone marrow aspiration was done from posterior superior iliac spine and sternum taking care of all the aseptic precautions. Smears were drawn and air dried from the material aspirated and stained. In the present study for scoring purposes the number and morphological changes were pre-defined before start of the study.

The bone marrow smears were examined as per the standard guideline and the findings were documented. The number of the megakaryocytes was considered as normal (one megakaryocyte per one to three low-power fields), increased (more than two megakaryocytes per low-power field) or decreased (one megakaryocyte per five to ten low-power fields).[2] 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). Dysplastic alterations were considered significant only when 10% or more of the megakaryocyte observed show the change.[1]

III. Results

Out of 50 cases of thrombocytopenia, 29 cases (58%) were observed in male and 21 cases (42%) were in female. Thrombocytopenia was most common in the age group of 21-30 years (52%; 26 cases) followed by that in 55-65 years of age group (48%; 24 cases).The most common cause of thrombocytopenia was ITP followed by megaloblastic anaemia & iron deficiency anaemia.

Dysplastic megakaryocytes in various haematological disorders are shown in table 1. ITP was most likely to have dysplastic megakaryocytes followed by megaloblastic anaemia & iron deficiency anaemia. Hypogranular forms were the most prevalent megakaryocytes followed by micromegakaryocytes. Hypogranular forms & micromegakaryocytes was most prevalent in megaloblastic anaemia followed by that in ITP. Budding, immature forms, bare nuclei & cytoplasmic vacuolization were observed in only 2 cases of ITP.Hypolobulation were observed in 9/17 cases of ITP followed by 7/12 cases of megaloblastic anaemia. Hyperlobulation were most prevalent in megaloblastic anaemia.

Table 2 shows average number of megakaryocytes per 10 LPF's in bone marrow aspiration. 82.3% cases of ITP had increased number of megakaryocytes followed by 66.7% cases of iron deficiency anemia & 58.3% cases of megaloblastic anaemia.

Table:1 Prevalence of dysplastic & non- dysplastic changes in various haematological disorders

Bone marrow impression	Dysplasia	Non- Dysplasia	Total
Megaloblastic Anaemia	5/41.7%	7/58.33%	12
ITP	6/35.3%	11/64.7%	17
Aplastic anaemia	3/75%	1/25%	4
Iron Deficiency anaemia	5/83.3%	1/16.7%	6
Dimorphic anaemia	2/100%	0/0%	2
AML	0/0%	1/100%	1
ALL	0/0%	1/100%	1
CML(Blast crisis)	2/100%	0/0%	2

Table: 2 Number per low-power field				
Bone marrow Impression	Normal	Increased	Decreased	Absent
Megaloblastic Anaemia	1/8.3%	7/58.3%	4/33.3%	0/0%
ITP	2/11.8%	14/82.3%	1/5.9%	1/5.9%
Aplastic anaemia	2/50%	1/25%	1/25%	00
Iron Deficiency anaemia	1/16.5%	4/66.7%	1/16.5%	0/0%
Dimorphic anaemia	0/0%	1/50%	1/50%	0/0%
AML	0/0%	1/100%	0/0%	0/0%
ALL	0/0%	1/100%	0/0%	0/0%
CML(Blast crisis)	1/50%	0/0%	1/50%	0/0%

Table 3: Morphological changes in megakaryocytes in various conditions								
Bone Marrow impression	Immature Megakaryocytes	Bare Megakaryocytic Nuclei	Cytoplasmic Vacuolization	Plaletlet Budding	Micromegakaryocytes	Hypogranular Forms	Hypolobulation of nucleus	Hyperlobulation of nucleus
Megaloblastic anaemia	00	01	00	00	06	07	07	03
ITP	02	02	02	02	02	06	09	01
Aplastic anaemia	00	00	00	04	04	01	00	01
Iron Deficiency anaemia	00	00	00	00	00	04	02	02
Dimorphic anaemia	01	01	02	02	02	02	01	01
AML	00	01	00	01	00	00	00	00
ALL	00	01	00	01	00	00	00	00
CML (blast crisis)	01	01	00	02	02	02	01	00

IV. Discussion

Out of 50 cases of thrombocytopenia, 29 cases (58%) were observed in male and 21 cases (42%) were in female similar to that observed by Choudhary et al.[1] Thrombocytopenia was most common in the age group of 21-30 years (52%; 26 cases) followed by that in 55-65 years of age group (48%; 24 cases). The most common cause of thrombocytopenia was ITP followed by megaloblastic anaemia & iron deficiency anaemia. In contrast the most common cause of thrombocytopenia in a study performed by Choudhary et al was megaloblastic anaemia followed by acute leukaemia & ITP.[1] According to the study of Muhury M et al the most common cause was AML, followed by ITP & ALL.[2]

Out of 17 cases of ITP 14(82.3%) had increased number of megakaryocytes in bone marrow aspiration smears similar to the observations of Choudhary et al[1]& Muhury M et al.[2] Dysplastic megakaryocytes were found in 6 cases(35.3%) similar to that found by Choudhary et al (21.2%) & in contrast to that found by Muhury M et al (89.5%)[1],[2]. The most morphological alteration found were hypolobulation (n=9,52.94%) & hypogranular forms (n=6,35.29%) followed by cytoplasmic vacuolation. This finding was in contrast to micromegakaryocytes observed by Shi Xd et al.[3]

Out of 12 cases of megaloblastic anaemia 7 cases(58.3%) had increased number of megakaryocytes. Dysplastic megakaryocytes were found in 5 cases(41.7%) similar to that found by Choudhary et al[1]& in contrast to that found by Muhury M et al[2]. The most common dysplastic changes were hypogranular forms(n=7,58.3%)%) & micromegakaryocytes (n=6,50%); in contrast to Choudhary et al & Muhury M et al[1],[2] who observed multiple separate nuclei to be the most common dysplastic feature.Out of 6 cases of iron deficiency anaemia, 4(66.7%)cases show increased number of megakaryocytes. 5 cases(83.3%) were observed to have dysplastic features; most commonly hypogranular forms(n=4,66.6%).

Out of 4 cases of aplastic anaemia in this study, dysplastic megakaryocyte was found in all the cases, the most common being micromegakaryocyte(n=4,100%)followed by hypogranular forms. These findings are similar to the observations of Choudhary et al[1] and in contrast to the findings of Tricot et al[4] who found normal morphology in all cases. 50% of the cases showed normal number of megakaryocytes; similar to that observed by Choudhary et al.[1]

Out of 2 cases of dimorphic anaemia, dysplastic changes were observed in 100% cases; the most common being hypogranular forms(100%) & micromegakaryocytes(100%) followed by cytoplasmic

vacuolization. The findings are similar to that observed by Tejinder Singh et al.[5]

Acute leukaemias (AML-Acute Myeloid Leukaemia & ALL-Acute Lymphocytic Leukaemia) show dysplastic megakaryocytes, most common being micromegakaryocytes followed by bare nuclei. The obeservations are similar to that observed by Choudhary et al & Muhury M et al.[1],[2] Acute leukaemias show increased number of megakaryocytes, similar to that observed by Choudhary et al.[1]

Out of 2 cases of CML (blast crisis), 50% show normal megakaryocytes per LPF's (low power field's)& 50% show decreased number. Both the cases show dysplastic features most common micromegakaryocytes & hypogranular forms. All these findings are similar to those observed by Tejinder Singh et al.[5]

V. Conclusion

The commonest cause of thrombocytopenia in this study was ITP followed by megaloblastic anemia & iron deficiency anaemia. In haematological disorders, dysplastic megakaryocytes were more commonly observed in ITP, megaloblastic anemia & iron deficiency anaemia. Due to the very common occurrence of dysplastic megakaryocytes in non-MDS haematological disorders, the threshold for dysplasia in megakaryocyte should be raised from the recommended 10%. However, further comparative study including cases of MDS has to be done to understand the significance of occurrence of the dysplastic megakaryocytes in non-MDS related thrombocytopenia.

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