

MEGAKARYOCYTIC ALTERATION IN CASES OF THROMBOCYTOPENIA- A BONE MARROW ASPIRATION STUDY

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ABSTRACT

BACKGROUND

Thrombocytopenia defined as platelet counts less than 1,50,000/ μ L is commonly found in many haematological practices. The various disorders ranges from benign to malignant conditions.

MATERIALS AND METHODS

All the bone marrow aspiration smears who presented with thrombocytopenia were retrospectively studied during the period May 2015 to April 2017. The study was conducted in the Department of Pathology, Regional Institute of Medical Sciences, Imphal. All the Leishman stained bone marrow aspirate smears were taken from the archives and studied again with special reference to the megakaryocyte number and the various morphology. The clinical details and the diagnosis were also noted.

RESULTS

A total of 258 bone marrow aspiration smears were included in the study. The most common cause of thrombocytopenia in the study was AML (53 cases), followed by nutritional anaemia (42 cases) and hypoplastic anaemia (38 cases). Various other causes like aplastic anaemia, infective cause and malignant infiltration of the bone marrow were also encountered. In cases of myelodysplasia, dysplastic forms, bare megakaryocytic nuclei, hypogranular forms and micromegakaryocytes were seen.

CONCLUSION

Giving an undue importance to the morphology and number of megakaryocytes, while reporting bone marrow aspiration smears can help in enabling the diagnostic accuracy of various haematological conditions.

KEYWORDS

Megakaryocytes, Thrombocytopenia, Bone Marrow.

HOW TO CITE THIS ARTICLE: Kutum P, Laishram RS, Devi KA, et al. Megakaryocytic alteration in cases of thrombocytopenia: A bone marrow aspiration study. J. Evid. Based Med. Healthc. 2017; 4(76), 4460-4464. DOI: 10.18410/jebmh/2017/889

BACKGROUND

Megakaryocytes (MK) developed from multipotent hemangioblast are responsible for the formation of platelets in the bone marrow and are then released in the blood. Platelets are formed from cytoplasmic buddings from megakaryocytes.^{1,2} Normal megakaryocytes develops in the bone marrow and reaches cell sizes <50-100 microns in diameter and ploidy ranging up to 128 N.^{3,4} The hallmarks of MK maturation includes endoreduplication (polyploidisation) and expansion of cytoplasmic mass.⁵

Financial or Other, Competing Interest: None.

Submission 01-09-2017, Peer Review 08-09-2017,

Acceptance 17-09-2017, Published 19-09-2017.

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DOI: 10.18410/jebmh/2017/889

Thousands of platelets from a single megakaryocyte are released following an complicated steps of remodelling. Abnormalities in this process can 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 1,50,000/ μ L) can lead to inadequate clot formation and increased risk of bleeding.⁶ Thrombocytopenia is commonly encountered in various haematological disorders including Myelodysplastic Syndromes (MDS) as well as various non-myelodysplastic haematological conditions.⁷ Dysplastic changes are well known in MKs in thrombocytopenia associated with MDS. However, they are also observed in Megakaryocytes (MK) in non-myelodysplastic haematological conditions, but scant data exist on the prevalence of dysplastic changes in MKs in them.⁶ The present study was undertaken to estimate the prevalence of various conditions associated with thrombocytopenia to record the megakaryocytic alterations in various cases of



thrombocytopenia for better understanding of the dysplastic features and their contribution to thrombocytopenia.

Aims and Objectives

To study the prevalence of various conditions associated with thrombocytopenia and to document the megakaryocytic alterations in various cases of thrombocytopenia.

MATERIALS AND METHODS

A retrospective study of bone marrow aspirates from patients with thrombocytopenia were conducted. The clinical details, complete blood counts and other relevant laboratory investigations were obtained. The bone marrow smears were stained with Leishman stain and examined for changes in the number and morphology of MKs in thrombocytopenia (<1,50,000/mm³). In the present study for scoring purposes, the number and morphological changes were predefined before start of the study. The cases were defined according to the work done by Muhury M et al⁷ and broadly the cases were tabulated accordingly. The number of the megakaryocyte was considered as- normal (1 MKs/1-3 low-power fields), increased (>2 MKs/low-power field) and decreased (1 MKs/5-10 low-power fields). The morphological changes of MKs that were studied included- nuclear segmentation, presence of immature forms, dysplastic forms, micromegakaryocytes, emperipolesis, platelet budding, cytoplasmic vacuolisation, bare megakaryocytic nuclei and hypogranular forms. The various morphological abnormalities of MK includes micromegakaryocytes, dysplastic forms, MKs with separated lobes and hypogranular forms. A MK with 4-16 nuclear lobes were considered normal. An immature MK was considered with the one lacking lobulation and scant bluish cytoplasm (Figure 1). Dysplastic MKs- Those with single/multiple separate nuclei. Micromegakaryocytes- MKs size- large lymphocyte/monocyte, single/bilobed nucleus.

The MKs were considered to show platelet budding if there was budding of cytoplasmic processes from their surfaces. Hypogranular forms- MKs with pale grey or watery clear cytoplasm, sparse or no granule. The no and morphology of the MKs in non-MDS related thrombocytopenia were assessed. Their significance was studied by comparing with the morphological changes in MDS. The sensitivity and specificity for those morphological features, which were significant in the relevant haematological disorders were also calculated.

RESULTS

A total of 258 of bone marrow aspirates from patients with thrombocytopenia were studied. Causes of thrombocytopenia for which bone marrow examination were sought and shown in Table 1. The most common cause of thrombocytopenia was encountered in Acute Myeloid Leukaemia (AML) (53 cases, 20.54%) followed by nutritional anaemia (42 cases, 16.27%) and hypoplastic anaemia (38 cases, 14.72%).

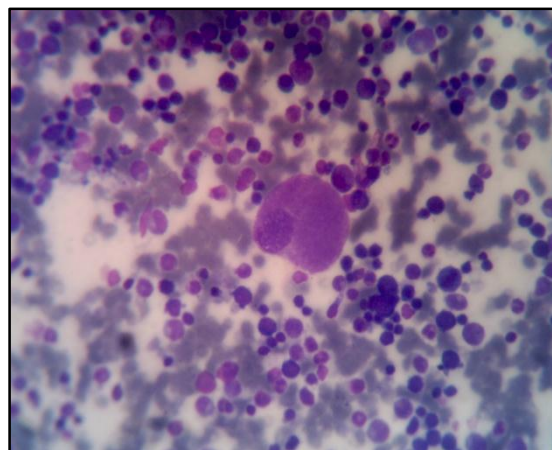


Figure 1. Photomicrograph of Bone Marrow Aspirates Showing a Hypolobated Megakaryocyte (Leishman Stain 10X)

Table 2 shows the various changes in number and morphology of megakaryocytes in various haematological conditions. AML shows a decrease in MK (46 cases), absent MK in 3 cases and 4 cases showed normal number of MK. Table 3 shows the morphological changes of megakaryocytes in different haematological disorders causing thrombocytopenia.

There was an increase in the number of MKs in 19 cases (94.7%) of ITP and immature MKs were seen in all the cases (sensitivity = 100%, specificity = 60%). Dysplastic forms were seen in 14 cases (70%), bare megakaryocytic nuclei in 16 cases (84%) and micromegakaryocytes in eight cases (42.1%) of ITP, their sensitivity being 89%, 84% and 42% respectively and specificity being 52%, 58% and 84%, respectively.

In cases of IAT, immature MKs were observed in all the five cases (sensitivity = 100%, specificity = 61%) and cytoplasmic vacuolisation was seen in two of the cases (sensitivity = 40%, specificity = 86%). In cases of MDS, dysplastic forms, bare megakaryocytic nuclei and micromegakaryocytes were seen. However, finding of micromegakaryocytes was most significant when compared to non-MDS causes of thrombocytopenia (specificity - 83%). Decreased platelet budding and absence of cytoplasmic vacuoles were also noted.

Sl. No.	Bone Marrow Diagnoses	No.	%
1.	AML	53	20.54
2.	NA (IDA and megaloblastic anaemia)	42	16.27
3.	Hypoplastic anaemia	38	14.72
4.	Reacting marrow with thrombocytopenia	32	12.4
5.	ALL	28	10.8
6.	MDS	25	9.68
7.	ITP	20	7.75
8.	ACD	6	2.32
9.	Metastatic deposits	6	2.32
10.	IAT	5	1.93
11.	CML (blast crisis)	2	0.77
12.	Drug induced	1	0.38

Table 1. Various Condition with Thrombocytopenia

AML- Acute myeloid leukaemia; NA- Nutritional anaemia; IDA- Iron deficiency anaemia; ALL- Acute lymphoblastic leukaemia; MDS- Myelodysplastic syndrome; ITP- Immune

thrombocytopenic purpura; ACD- Anaemia of chronic disease; IAT- Infection-induced thrombocytopenia; CML- Chronic myeloid leukaemia.

Sl. No.	Bone Marrow Impression	Number of Megakaryocytes per Low Power Field			
		Normal	Increased	Decreased	Absent
1.	AML	4	0	46	3
2.	NA (IDA and megaloblastic anaemia)	27	2	12	1
3.	Hypoplastic anaemia	0	0	35	3
4.	Reacting marrow with thrombocytopenia	21	7	4	0
5.	ALL	2	1	23	2
6.	MDS	11	3	11	0
7.	ITP	1	19	0	0
8.	ACD	4	0	2	0
9.	Metastatic deposits	0	0	6	0
10.	IAT	1	0	4	0
11.	CML (Blast crisis)	0	0	2	0
12.	Drug Induced	0	0	1	0

Table 2. Incidence of Changes in Number of Megakaryocytes in Different Haematological Disorders Causing Thrombocytopenia

Bone Marrow Impression	Immature	Dysplastic	Bare Nuclei	Emperipolesis	Platelet budding	Cytoplasmic vacuolation	Micro	Hypogranular	Number of Nuclear Lobes	
									Normal	Decrease
AML	8	16	4	6	15	0	10	1	48	5
NA (IDA and megaloblastic anaemia)	6	11	9	7	14	3	8	2	36	0
Hypoplastic anaemia	4	6	2	1	6	1	7	3	8	0
Reacting marrow with thrombocytopenia	8	2	1	1	30	0	4	3	28	0
ALL	3	5	4	1	10	2	3	0	23	0
MDS	12	20	16	8	4	0	15	1	20	0
ITP	20	16	14	13	15	9	8	1	18	2
ACD	4	1	0	1	5	2	1	0	5	0
Metastatic deposits	2	0	0	0	4	0	0	2	6	0
IAT	5	1	0	1	5	2	0	0	4	1
CML (Blast crisis)	1	1	0	0	1	0	1	0	2	0
Drug Induced	0	0	0	0	1	0	0	0	0	01

Table 3. Morphological Changes of Megakaryocytes in Different Haematological Disorders Causing Thrombocytopenia

DISCUSSION

Normal maturation and platelet formation results from megakaryocytic Deoxyribonucleic Acid (DNA) replication that occurs without cell division resulting in large lobulated, polypoid nucleus. A wide variety of growth factors like thrombopoietin act synergistically with other haematopoietic cytokines and transcriptional factors stimulating the maturation and growth of MKs.⁸ A defect in any of the stages of megakaryocytopoiesis can lead to dysmegakaryocytopoiesis and thrombocytopenia.

A shift to young, immature, less polypoid MKs and fewer mature platelet-producing MKs was the outstanding morphological feature noted in all the cases of ITP in the present study (sensitivity = 100%, specificity = 68%). Similar findings were observed by Houwerzijl et al⁹ wherein they said it is because of apoptotic and para-apoptotic type of programmed cell death of mature megakaryocytes. Most of the abnormal MKs were surrounded by neutrophils and macrophages, some in the state of phagocytosis.

Inappropriate PCD of mature MKs can disrupt platelet formation and apoptosis-like PCD (para-apoptosis) occurs in ITP. This finding is especially useful when some patients of MDS present with isolated thrombocytopenia, thus mimicking ITP. Dysplastic forms were seen in 16 cases (80%), bare megakaryocytic nuclei in 14 cases (70%) and micromegakaryocytes in 8 cases (40%). Their sensitivity being 86%, 80% and 42%, respectively and specificity, 52%, 58% and 84%, respectively.

Emperipolesis, seen in 13 of the 20 cases of ITP (65%; sensitivity = 68%, specificity = 74%) with lymphocytes in five and lymphocytes along with nucleated red blood cells in four cases correlated with that of Roznan and Vives-Corrans.¹⁰ The cytoplasmic vacuolisation seen in nine cases (47.4%), which ultrastructurally represents mitochondrial swelling was also observed by Levine¹⁰ and Houwerzijl et al⁹ and this reflects an increased megakaryocyte turnover and indicates degenerative changes such as those of apoptosis and para-apoptosis. Another plausible explanation for the

cytoplasmic vacuolisation is autophagy to maintain cell metabolism when there is increased metabolic demand and nutrition deficiency due to increased megakaryocytopoiesis or it might be a way of sequestration and degradation of specific pathogens such as immunoglobulins.

All the above features suggested dysmegakaryocytopoiesis, which largely contributes to diminished platelet production. The fact that some patients respond to thrombopoietin mimetics further supports the fact that suppression of platelet production is an important mechanism in some ITP patients. The antiplatelet autoantibodies to glycoprotein IIb/IIIa and Ib/IX seen in ITP interfere with platelet production and release by causing megakaryocyte destruction and abnormal maturation.¹¹⁻¹³ The destruction of megakaryocytes is either by phagocytic cells or by activation of complement or by induction of apoptosis.¹³ They also inhibit megakaryocyte colony formation and proplatelet formation, the defect being in the common erythroid megakaryocyte stem cell. Therefore, altered megakaryocytic morphology and destruction resulting in defective platelet production and immune-mediated platelet destruction together contribute to thrombocytopenia.¹² The fact that some patients respond to thrombopoietin mimetics, further supports the fact that suppression of platelet production is an important mechanism in some ITP patients.¹³

In 19 of the 20 cases of ITP, there was an increase in the number of MKs, which was also observed by George, Harake, Raskob¹⁴ and Levine.¹⁰ This was attributed to the stimulation of the marrow MKs to synthesise platelets at an increased rate due to the immune-mediated platelet destruction in the spleen and other reticuloendothelial tissues. The severity of thrombocytopenia with increased megakaryocytopoiesis correlates with increased Mean Platelet Volume (MPV) in patients with ITP. A low MPV is observed in myeloproliferative disorders, hypersplenism and thrombocytopenia associated with sepsis.¹⁵

All five cases of IAT had increased MKs as noted by Alter, Scanlon and Schechter.¹⁶ The microbes might directly damage the platelets or alter them to become antigenic, resulting in specific antiplatelet antibody formation. Alternatively, a virus-antivirus complex could precipitate on the platelets and damage them resulting in compensatory increase of megakaryocyte in the bone marrow.¹⁵

Immature MKs were observed in all the five cases (sensitivity = 100%, specificity = 61%) similar to that of Meindersma and de Vries,¹⁷ which was considered to be due to the increased megakaryocyte turnover. Cytoplasmic vacuolisation was seen in two of the cases (sensitivity = 40%, specificity = 86%) correlated with that of Channarin and Walford.¹⁸ It was pointed out that in acquired cytomegalovirus infection, this was due to toxic change caused by the virus. Recent studies have shown immune-mediated platelet destruction to be the cause of thrombocytopenia in human immunodeficiency virus, hepatitis C virus and *Helicobacter pylori* infections.¹⁹

MKs were decreased or absent in aplastic anaemia, which was also observed by Shaddock.²⁰ This was attributed

to bone marrow suppression and significant inhibition of nucleic acid synthesis in the MKs. The hypolobated forms and dysplastic forms seen were in contrast to those of Tricot et al²¹ where MKs were of normal morphology. The dyspoietic megakaryocyte, which was present in one case of aplastic anaemia showed that the patient was a known case of ITP who had subsequently developed aplastic anaemia and these abnormal MKs had persisted in the marrow. Presence of stem cell defect in ITP patients can progress to overt marrow failure.

According to Lim and Ifthikaruddin²² along with immune-mediated platelet destruction, there is decreased platelet production when the marrow is involved by lymphoma. Bone marrow suppression by chemotherapeutic agents and platelet sequestration in the spleen also contribute to thrombocytopenia in lymphoma.

AML in the present study, 53 cases (20.54%) was the most common cause of thrombocytopenia for which bone marrow examination was sought. In 46 of the cases (86%), the number of MKs was decreased with 3 cases not showing any MKs. Bone marrows of AML were tightly packed with leukaemic blast cells with maturation arrest as was observed by Tricot et al also.²¹

In megaloblastic anaemia, dysplastic forms were seen in nine cases (75%; sensitivity = 75%, specificity = 49%), MKs with separation of nuclear lobes and nuclear fragments were observed, which was also observed by Wikramasinghe.²³ This was attributed to diminished DNA synthesis leading to nuclear maturation defect.

Dyspoietic MKs are well described in MDS, however, limited literature exists regarding their significance in non-MDS cases. In the present study, there was no significant difference in the dyspoietic features in non-MDS cases as compared to MDS cases, except for the micromegakaryocytes ($P < 0.05$) with specificity of 83% in MDS. The micromegakaryocytes represent abnormal MKs that have lost their ability to undergo endomitosis, a qualitative defect of megakaryocyte maturation. Increased number of abnormal MKs, particularly the micromegakaryocytes suggest an expansion of megakaryocytic precursors, an arrest in terminal megakaryocyte differentiation and impaired nuclear development.²⁴ Hence, dyspoietic features by themselves do not specify MDS, other haematological conditions causing thrombocytopenia have to be considered in the differential diagnosis.

CONCLUSION

Evaluating the changes in the number and morphology of megakaryocytes while reporting bone marrow aspiration smears might provide better understanding of the pathogenesis of various haematological conditions with thrombocytopenia.

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