

ORIGINAL ARTICLE

Megakaryocytic Characteristics in Diagnosis of Patients with Thrombocytopenia

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ABSTRACT

Background: The morphology of megakaryocytes plays a major role in thrombopoiesis. Dysmegakaryocytopoiesis and thrombocytopenia can result from imperfection in any phase of megakaryocytopoiesis. The structure, morphology, and characteristics of megakaryocytes can differentiate and identify the etiology of underlying diseases such as leukaemia, mixed anaemia, idiopathic, aplasia, or infiltration. This research aimed to comprehend megakaryocyte attributions and their contribution to the diagnosis of thrombocytopenia.

Methods: This was a cross-sectional analysis of all successive cases of thrombocytopenia bone marrow aspirates over 12 months (January 2019 to December 2019) at the Department of Pathology, Indus Medical College Hospital Tando Muhammad Khan. With a 100X lens, megakaryocyte morphology was studied. Data were analysed by SPSS and Chi-square was performed to find correlation at the 95% confidence interval.

Results: Among the 91 subjects of thrombocytopenia, the commonest etiology of thrombocytopenia was idiopathic thrombocytopenia 34(37.36%). The common morphological changes in megakaryocytes were hypolobated megakaryocytes and bare megakaryocyte nuclei. Megakaryocyte numbers were increased for 31 (91.17%) patients among idiopathic thrombocytopenia, though the megakaryocyte numbers were decreased among 19 (90.47%) patients having acute leukaemia. A change to immature, young, less polyploid and bare nuclei forms was observed in immune thrombocytopenia. Immature types were seen in 15 cases, 14 cases of bare megakaryocytic nuclei, 15 cases of hypolobation and one case of micromegakaryocytes.

Conclusion: Many similarities among various haematological diseases were observed in megakaryocytic morphology. However, in megakaryocytic thrombocytopenia, high megakaryocyte counts and evidence of bare nuclei of megakaryocytes ($p=0.02$) and hypolobated types ($p=0.03$) proved important and play a very essential role in diagnosis.

Keywords: Megakaryocytes; Thrombocytopenia; Bone Marrow Aspirate; Diagnosis; Dysmegakaryocytopoiesis.

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INTRODUCTION

The megakaryocytes derive from the pluripotent hematopoietic stem cells, which go through the involvement of lineage, differentiation, and proliferation under the control of cytokines, specifically thrombopoietin (TPO) to produce

1000–3000 platelets¹. This is evident that the morphology of megakaryocytes takes an essential part in thrombopoiesis and those additional platelets than smaller platelets are produced by giant megakaryocytes. The higher the platelet output, the greater the ploidy (nuclear lobulation) will happen. The processes that regulate the size of

platelets are incompletely known but it appears that the body is trying to the preservation of total mass of the platelets and not a total count of the platelet. When multiplying the overall count of platelets by mean platelet volume, the total mass of platelets is constantly extracted¹.

As the count of platelets reduces, mean platelet volume enhances. When the peripheral smear reveals megathrombocytes (giant platelets), it typically suggests peripheral platelet intake. Platelets play critical roles in daily life restoration of minute vascular damage, repair of the wound, the response of the innate immune system and biology of metastatic cell tumours. The mean number of platelets in humans varies from $150-350 \times 10^9/L$, although the amount is kept within reasonably narrow limits for any person. The replication of deoxyribonucleic acid (DNA) of megakaryocytes that happens devoid of cell division results in normal maturation and platelet formation, cause a giant lobulated polyploid nucleus. Wide range of growth factors, such as the thrombopoietin, function concurrently with other cytokines of haematopoiesis and factors of transcription that stimulate megakaryocyte growth and maturation. Dymegakaryocytopoiesis and thrombocytopenia may lead to a fault in either phase of megakaryocytopoiesis².

Thrombocytopenia is characterized as less than $1, 50, 000/mm^3$ of platelet counts. It is the source of bleeding that is most common. Thrombocytopenia arises from only four mechanisms, considering the amount and variety of disorders that are etiologically related: artifactual thrombocytopenia, low development of platelets, rapid degradation of platelets and an unequal distribution or platelet pooling inside the body³. Changes in the morphology of megakaryocytes in immune thrombocytopenic purpura, e.g., wide spread vacuolization of cytoplasm, hypogranularity and smoothing of the cell membrane, were previously identified by Frank in 1915 and further verified by other researchers. This was claimed that certain changes were artefacts caused via techniques of staining or fixation¹.

Changes of dysplasia in thrombocytopenia affiliated to a myelodysplastic syndrome in megakaryocytes are well documented. Non-myelodysplastic haematological disorders, such as immune thrombocytopenic purpura, aplastic anemia, hypersplenism, acute myeloid leukaemia, acute lymphoblastic leukaemia, leukaemia lymphoma syndrome, metastasis of bone marrow, blast crisis of chronic myeloid leukaemia is often found in megakaryocytes. However, there is no evidence on the occurrence of megakaryocyte dysplastic shifts in non-myelodysplastic haematological conditions². Study in 1995 found that myelodysplastic syndrome is distinguished by the occurrence of atypical micromegakaryocytes⁴. By 2009, idiopathic

thrombocytopenic purpura (ITP) was proved to have increased megakaryocyte number with immature forms². Study in 2010 revealed megakaryocytes in megaloblastic anaemia were hyperlobated with finely stippled chromatin⁵. A 2012 study similarly postulated that immune-mediated thrombocytopenic purpura is distinguished via major morphological changes in bone marrow megakaryocytes⁶.

Additional researches on the assessment of megakaryocytic modification along with its relation with thrombocytopenia may give pathogenesis awareness regarding various haematopoietic problems that can recognise the wider clinical implementation of the recent strategies for controlling function and control of platelet. This research aimed to identify changes in the morphology and number of megakaryocytes among various haematological disorders that cause thrombocytopenia as well as determining if certain modifications had an important connection to various etiologies of thrombocytopenia.

METHODS

It was cross-sectional research carried out at Indus Medical College Hospital, Tando Muhammad Khan, with individuals of thrombocytopenia undergone bone marrow aspirates for 1 year period (January 2019 to December 2019). The study was conducted after approval from Ethical Board Review of the Institution (No.ERB/IMC/2018-026). The research included all participants with thrombocytopenia for which bone marrow aspiration was performed. Cases that met the thrombocytopenia but lacked a characteristic bone marrow aspirate were removed from the study. The subjects received informed consent and retained confidentiality during the study. Clinical information such as history, physical observations, peripheral blood smear, full blood counts and other necessary related parameter studies were assessed. The peripheral smears were organized and stained by using Giemsa stain as per standard guidelines⁷. Bone marrow aspiration was done from the posterior superior iliac spine in subjects having thrombocytopenia and then stained with Giemsa, followed by examination under the microscope. Rating of megakaryocytes was organized as normal megakaryocytes: one megakaryocyte/1-3 low-power fields, increased megakaryocytes: >two megakaryocytes/low-power field, or decreased megakaryocytes: one megakaryocyte/5-10 low-power fields¹.

The characteristics of megakaryocytes were evaluated with microscopy (100x)⁸, including immature structure/form, segmentation of nucleus, dysplasia, micromegakaryocytes, budding of the platelets, vacuolization in the cytoplasm, bare nuclei of megakaryocyte, hypolobulation, and hypogranulation.

Dysmegakaryopoiesis is the presence of irregular megakaryocytes comprising dysplasia, micromegakaryocytes, megakaryocytes having split lobes and hypogranulation. It is postulated that typical megakaryocytes have 4-16 lobes of nucleus. Megakaryocytes with immaturity were classified as young megakaryocyte types. They usually have slight cytoplasm with a bluish colour and lack of lobes of the nucleus occupying most of the cell. Megakaryocytes having single or multiple distinct nuclei are known as dysplastic megakaryocytes. The micromegakaryocytes are classified as megakaryocytes with size as that of the single/bilobed nucleus of a large lymphocyte/ monocyte. If cytoplasmic processes emerge from their surfaces, megakaryocytes are supposed to exhibit budding of platelets. Megakaryocytes having water clear or pale grey cytoplasm and no or light granules are known as hypogranular types. It is also known as the form of cell observed in emperipolesis inside megakaryocyte. Morphology and the number of megakaryocytes with thrombocytopenia were subsequently evaluated.

The data collected were tested for totality, entered, and analyzed by SPSS. The statistics with descriptive nature were prepared using charts and statistical

tests (e.g., Chi-squared test) were performed to find correlation of megakaryocyte morphology and various causes of thrombocytopenia at the 95% confidence interval.

RESULTS

Among all 91 bone marrow aspirate samples, thrombocytopenia was present in groups of all ages; though most subjects were younger than 30 years (64.83%). Similarly, females (56.04%) were more affected than males. The age was ranged between 6 to 50 years. A 48 (52.74%) were males while 43 (47.25%) were females, 64 (70.32%) were >12 years of age while 27 (29.67%) were <12 years of age. Bleeding tendency (n=31; 34.06%) followed by pallor (n=29; 31.86%) was commonest clinical characteristic observed. In acute leukaemia, the typical clinical characteristics were fever (92.3%), pallor (68.13%) and organomegaly (64.83%), commonest etiology of thrombocytopenia for which aspiration of bone marrow was pursued was idiopathic thrombocytopenia (37.36%), followed by acute leukaemia (24.17%), as shown in Figure 1.

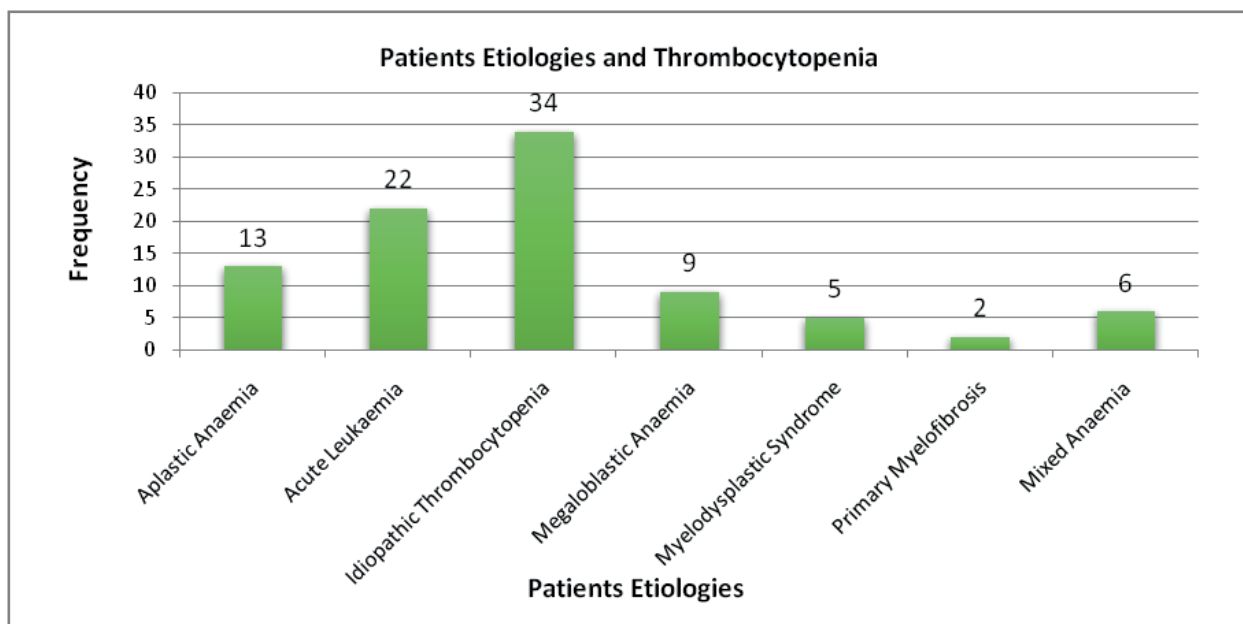


Figure 1: Etiologies in patients with thrombocytopenia.

As shown in Table 1, megakaryocyte numbers were increased for 31 patients (91.17%) among idiopathic thrombocytopenia, though the megakaryocyte numbers were decreased among 19 patients (90.47%) having acute leukaemia. By observing the characteristic megakaryocyte morphology, particular features of morphology observed among all

patients having idiopathic thrombocytopenia in the current research was a change to immature, young, less polyploid and bare nuclei forms. Immature types were seen in 15 cases, 14 cases of bare megakaryocytic nuclei, 15 cases of hypolobation and one case of micromegakaryocytes.

Table 1: Megakaryocyte among various etiologies of thrombocytopenia.

Cause of Thrombocytopenia	Megakaryocytes per Low Power Field		
	Normal (Megakaryocytes/1-3 Low Power Fields)	Increased (>2 Megakaryocyte/Low Power Fields)	Decreased (Megakaryocyte/5-10 Low Power Fields)
Aplastic Anaemia	01	0	12
Acute Leukaemia	03	0	19
Idiopathic Thrombocytopenia	03	31	0
Megaloblastic Anaemia	09	02	0
Myelodysplastic Syndrome	05	0	0
Myelofibrosis	0	02	0
Mixed Nutritional Anaemia	0	06	0
Total	21	41	31

In Table 2, the morphology observed was megakaryocyte count are higher than for other tabulated. The chances of an increased idiopathic thrombocytopenia (Table 3).

Table 2: Megakaryocyte morphology as per etiologies of thrombocytopenia.

Etiologies	ITP	AA	AL	MBA	MDS	MF	MNA	Total
Immature MK	15	02	04	01	03	0	05	30
Dysplastic MK	03	0	03	05	05	0	03	19
Bare MK Nuclei	23	02	02	03	03	01	04	38
Emperipolesis	02	0	02	0	02	0	0	06
Platelet Budding	03	0	01	03	0	0	01	08
Cytoplasmic Vacuolation	02	0	02	02	02	0	0	08
Micro MK	04	0	0	03	0	0	01	09
Hypogranular	01	02	03	01	02	0	04	13
Hypolobated MK	17	01	01	02	01	01	02	24

ITP = Idiopathic thrombocytopenia purpura, AA = Aplastic anaemia, AL = Acute Leukaemia, MBA =Megaloblastic anaemia, MDS = Myelodysplastic syndrome, MF = Myelofibrosis, MNA = Mixed Nutritional Anaemia, MK = Megakaryocyte

Table 3: Association of cause of thrombocytopenia and megakaryocytes.

Cause of Thrombocytopenia	Number of Megakaryocyte		p-Value
	Increased	Normal	
Idiopathic	31	03	<0.001
Others	10	18	0.02
Morphology	Cause of Thrombocytopenia		p-Value
	Idiopathic	Others	
Bare Megakaryocytic Nuclei	23	15	0.02
Hypolobulated Forms	17	07	0.03

DISCUSSION

In the current study, thrombocytopenia was observed with an average age of 27.18 years. ITP was the most common cause. Acute leukaemia accompanied by aplastic anaemia was the next commonest etiology; myelofibrosis and megaloblastic anaemia was the least common. The morphological features and number of megakaryocytes correlated to various patients of thrombocytopenia may be observed by Giemsa-stained smears of bone marrow aspirate.

For a wide variety of haematological disorders, this may enhance diagnostic precision, thereby allowing adequate therapeutic strategies².

Acute myeloid leukemia (AML) was commonest reason for thrombocytopenia. Idiopathic thrombocytopenia purpura (ITP) followed by dimorphic anaemia and acute lymphoblastic leukaemia (ALL) was next commonest cause². Though in our study, ITP was the commonest cause followed by acute leukaemia. Megakaryocyte number was increased in our research, which was

found by George et al.²⁶ and Levine²⁷ as well. The relation was with activation of megakaryocytes in marrow to synthesize platelets at accelerated speed due to immune-mediated destruction of platelets^{9,10}. Wang and Shen observed the thrombocytopenia severity with enhanced megakaryocytopoiesis correlated to elevated mean platelet volume (MPV) among ITP patients⁹.

In study by Muhury et al., number of bone marrow megakaryocytes was elevated in 18 of 19 cases with ITP, like the current study. Glycoprotein Ib/IX and IIb/IIIa antiplatelet autoantibodies observed in ITP compromise the platelet development and liberation by triggering the degradation and abnormal maturation of megakaryocytes^{2,10,11}. They found over-proliferation of bone marrow megakaryocytes (MKs) along with abnormal characteristics of megakaryocytes in most patients with ITP by Hu et al.¹². In the study by Dameshek and Miller, megakaryocytes were elevated in bone marrow of acute and chronic ITP, while circulating platelets in blood were uncommon, the finding analogous to current research^{13,28}. Development of platelets was significantly increased following splenectomy, which was seen in 69-81%; huge masses of fresh marrow platelets were also very remarkable.

Results of an increase in megakaryocytes along with substantial decrease in production of platelets in marrow prior to splenectomy and a pronounced rise in manufacturing of platelets following splenectomy suggest explicit pathogenetic association between spleen and disease¹³. Characteristic morphology of megakaryocytes in ITP was similar with results by Nurden²⁹, in which they said it is due to mature megakaryocytes' para – apoptotic and apoptotic variety of programmed cell death. Improper programmed cell death of mature megakaryocytes may interrupt the production of platelets and ITP occurs with para-apoptosis form of programmed cell death. This result is particularly beneficial when isolated thrombocytopenia is present in some myelodysplastic syndrome (MDS) patients, thus mimicking ITP¹.

Emperipolesis was found in a research performed by Rozman and Vives-Corrons among 1 of 17 patients of IP with lymphocytosis¹⁸. In our study, emperipolesis was observed in 2 cases of ITP. Levine²⁷ and Houwerzijl et al. have observed vacuolization in cytoplasm in 1 case¹⁹. In our study, it was observed in 8 cases with majority of them having ITP and acute leukaemia. Autophagy to sustain cell metabolism is another possible reason for cytoplasmic vacuolization while metabolic demand is increased and nutrition deficiency is evident due to high megakaryocytopoiesis.

In ITP, the existence of bare megakaryocytic nuclei

was important. Likelihood of ITP was greater by bare nuclei of megakaryocytes than with other etiologies of thrombocytopenia. Bare nuclei of megakaryocytes were observed in 16 ITP patients (84.2%), in study conducted by Muhury et al.². In this analysis, megakaryocytes turned absent or reduced among patients with aplastic anaemia that was seen by de Masson et al.³⁰ as well. They related it to repression of the bone marrow as well as major inhibition in the megakaryocytes of synthesis of nucleic acid¹⁵.

Findings shown by Dameshek and colleague²⁸ also demonstrated that number of lymphocytes enhanced and that of megakaryocytes decreased in bone marrow among patients with aplastic anaemia that revealed failure of hematopoietic function. However, no any morphological anomalies occurred among the patients of bone marrow of aplastic anaemia^{13,16,20}. While young, naked, hypolobed and hypogranular structures were observed in present sample.

In aplastic anaemia, hypolobate, bare, immature, and dysplastic types with emperipolesis, vacuolization of cytoplasm, and budding of platelets of megakaryocytes were seen in research of Muhury et al.^{2,17,21}, similar findings as in our study. Though, in aplastic anaemia, Tricot et al. showed regular morphology of megakaryocytes^{23,23}. Similarly, not many cases of myelodysplastic syndrome, myelofibrosis, megaloblastic anaemia, and mixed nutritional anaemia along with idiopathic thrombocytopenia were included in this analysis for comparison. There were 5 MDS cases, all of which displayed a normal number of megakaryocytes in the bone marrow^{18,24,25}. Discovery of emperipolesis among anaemia was observed in Tavassoli study¹⁹. In the current research, though, dysplastic megakaryocytes and emperipolesis were very few, which may be because of lesser sample size. For cases of thrombocytopenia because of myelodysplastic or other etiologies, morphological changes of megakaryocytes are valuable and carry particular attention. Therefore, current study postulates that diagnostic accuracy of various etiologies of thrombocytopenia is enhanced by associating the various types of megakaryocytes found with the number of megakaryocytes present in the aspiration slide of the bone marrow. Therefore, for an extensive range of haematological conditions, knowledge of changes of morphology of megakaryocytes can enhance the diagnostic accuracy.

CONCLUSION

There are many similarities between different haematological diseases according to the morphological changes of megakaryocytes. However, a diagnostic approach can diverge the available comprehensive information. But in this research, idiopathic thrombocytopenia enhanced

the number and the existence of bare nuclei of megakaryocytes and hypolobulation which could be important for further investigations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The Ethical Review Board of the Institution had approved the study with the reference No.ERB/IMC/2018-026.

PATIENT CONSENT

As this was laboratory-based study, patients were only informed verbally.

AUTHORS' CONTRIBUTION

SAJ had given the study concept, performed experimentation, and written the discussion. HR performed the data analysis while NN interpreted the results. RNS had also contributed in the data analysis and written the literature review. SIK assisted in experimentation and RAT also helped in literature review writing.

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